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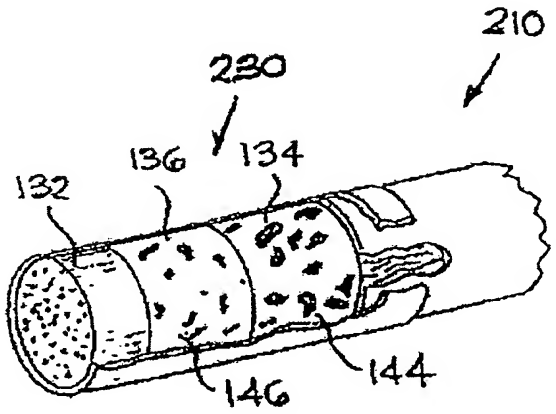

FIG. 1

(57) Abstract: Reduced risk tobacco-related products and methods of use. These tobacco-related products (e.g., cigarettes or filters) are designed to reduce the biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to the amount of biological insult induced by another cigarette, such as a conventional or reference cigarette (e.g., 2R4F), in human cells, and to provide a reduced risk cigarette that meets a cigarette smoker's sensory/perception needs.

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REDUCED RISK TOBACCO PRODUCTS AND USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to US Provisional Application Number 60/976,291, filed September 28, 2007, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] The present invention relates to the development of reduced risk cigarettes and methods of use thereof. More particularly, aspects of the invention concern cigarettes that are designed to reduce the induction of biological insult, for example, DNA double strand breaks (DNA DSBs), cell death or perturbation of RNA transcriptome or proteome in human. Another aspect concerns approaches to gradually reduce the presence of a toxicant in cigarette smoke while adjusting a cigarette smoker's sensory/perception needs.

Background

[0003] Toxicants in tobacco smoke induce several biochemical changes in human cells. Cigarette smoke contains over 4,000 chemicals. At present, the International Agency for Research on Cancer (IARC) concludes that there are at least 81 possible, probable, or proven human carcinogens in tobacco smoke (International Agency for Research on Cancer, Tobacco Smoking and Involuntary Smoking: Monograph on the Evaluation of Carcinogenic Risk of Chemicals to Humans. Vol. 83, 2003, Lyon, France). These include nicotine, tar, and carbon monoxide, as well as formaldehyde, ammonia, hydrogen cyanide, and arsenic, for example. Cigarette smoke is also known to cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome in cells that contact the cigarette smoke (e.g., cells of the oral cavity and lungs), which may lead to adverse health consequences. Despite the health risk associate with smoking, people continue to consume cigarettes.

[0004] Many companies have developed various products that have a reduced level of toxicants delivered in cigarette smoke but very few products have had measurable success. Historically, cigarette smokers have associated "reduced risk" or "reduced nicotine" or "low tar" cigarettes with a lack of satisfaction and poor taste. For example, Phillip Morris

introduced large carbon length filters and documented that an increase in the length of the carbon filter decreased the acceptance based on taste. Gaworski CL. SCoR Program. Carbon filter technology. Philip Morris USA. Powerpoint Meeting presentation at Massachusetts Department of Health, February 7, 2004.

[0005] Many products in this class of cigarettes achieve a low level nicotine delivery by using expanded, puffed tobacco in the rod accompanied by significant filter ventilation. The response to these types of products has been one of general dissatisfaction with taste and, oftentimes, compensatory behavior (e.g., blocking of ventilation holes or longer, deeper inhalation) is developed in order to improve the taste and/or delivery of nicotine.

[0006] Accordingly, cigarette smokers do not readily switch from their favorite brand to reduced risk cigarettes because the reduced risk cigarettes do not fulfill the tobacco user's sensory/perception needs. There remains a need for reduced risk cigarettes, particularly products that fulfill a tobacco user's sensory perception needs (*e.g.*, those that have an agreeable taste and/or odor).

SUMMARY OF THE INVENTION

[0007] In one embodiment, a cigarette comprises a blend of cured tobacco, wherein the blend comprises a non-transgenic Burley tobacco and a non-transgenic Flue-cured or Bright tobacco, wherein the non-transgenic Burley tobacco is present in an amount of about 45-70% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco; and the non-transgenic Flue-cured or Bright tobacco is present in an amount of about 55-30% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco.

[0008] In another embodiment, a cigarette comprises a blend of cured tobacco, wherein the blend comprises a non-transgenic Burley tobacco and a non-transgenic Flue-cured or Bright tobacco, wherein the non-transgenic Burley tobacco is present in an amount of about 85-92% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco; and the non-transgenic Flue-cured or Bright tobacco is present in an amount of about 8-15% by weight based on the

combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco.

[0009] The non-transgenic Burley tobacco may be a low alkaloid variety such as LA Burley 21, and/or the non-transgenic Flue-cured or Bright tobacco may be a low alkaloid variety. In one embodiment, the cigarette described herein comprises the non-transgenic Burley tobacco that is present in the amount of about 50% by weight, and the non-transgenic Flue-cured or Bright tobacco that is present in the amount of about 50% by weight. In another embodiment, or the cigarette described herein comprises the non-transgenic Burley tobacco that is present in the amount of about 90% by weight, and the non-transgenic Flue-cured or Bright tobacco that is present in the amount of about 10% by weight.

[0010] The cigarette may further comprise expanded stem tobacco or Oriental tobacco.

[0011] In another embodiment, the cigarette is configured to produce a mainstream smoke that generates reduced biological insult, for example, DNA double strand breaks (DSBs), cell death or perturbation of RNA transcriptome or proteome in lung cells than the mainstream smoke from a 2R4F reference cigarette under the same smoking conditions.

[0012] Another aspect of the invention is directed to a filter, and the cigarette described above comprising a filter, where the filter comprises a carbon and/or a weak base amine-containing resin. A carbon may be used in the filter that has a total pore volume of from 0.1 mL/g to 0.9 mL/g. In another embodiment, a certain percentage of the carbon has a pore volume distribution of 0.1 mL/g to 0.9 mL/g, wherein the percentage of carbon having the pore volume distribution is least about 50%. In another embodiment, the carbon has an average pore diameter of 0.6 nm to 1.1 nm.

[0013] In one embodiment, the filter comprises about 30-100 mg of the carbon. In another embodiment, the filter comprises an activated carbon having an activity of 50-60. In a further embodiment, carbon is TA95 activated carbon.

[0014] In another embodiment, the filter comprises about 10-50 mg of the weak base amine-containing resin. In a further aspect the weak base amine-containing resin contains at least or equal to about 1.3-1.5%, such as 1.4% nitrogen atoms (N) in the form of amine functional groups. In one embodiment, all of the nitrogen atoms are in the form of

primary amine functional groups, and in other embodiments the nitrogen atoms are in the form of a mixture that includes primary amine functional groups. In another embodiment, the ratio of the carbon to the weak base amine-containing resin in the filter is from about 1:1 to 4:1.

[0015] The filter may also comprise sepiolite. The filter may also be configured in a tripartite design comprising three compartments for containing each of the carbon, the weak base amine-containing resin, and the sepiolite.

[0016] Another aspect is directed to a cigarette filter comprising a carbon that has a total pore volume of 0.1 mL/g to 0.9 mL/g, or about 0.3 mL/g to about 0.7 mL/g, or about 0.4 mL/g to about 0.6 mL/g, and/or having a certain percentage of the activated carbon having a pore volume distribution of 0.1 mL/g to 0.9 mL/g, wherein the percentage of carbon having the pore volume distribution is least about 50%; and optionally a carbon having an average pore diameter of 0.6 nm to 1.1 nm.

[0017] The cigarette filter may further comprise a weak base amine-containing resin, such as one in which the ratio of the carbon to the weak base amine-containing resin in the filter is from about 1:1 to 4:1. The cigarette filter may comprise about 30-100 mg of the carbon. In one embodiment, the carbon is an activated carbon having an activity of 50-60. In one embodiment, the activated carbon is TA95.

[0018] The cigarette filter may also comprise about 10-50 mg of weak base amine-containing resin. The weak base amine-containing resin may contain at least or equal to about 1.3-1.5%, such as 1.4% nitrogen atoms (N) in the form of amine functional groups. In one embodiment, all of the nitrogen atoms are in the form of primary amine functional groups, and in other embodiments the nitrogen atoms are in the form of a mixture that includes primary amine functional groups.

[0019] The cigarette filter described above may also further comprise sepiolite. The filter described above may have a tripartite design comprising three compartments for containing each of the carbon, weak base amine-containing resin, and sepiolite. Filters described above may be used in a cigarette.

[0020] One aspect is directed to method of making a filtered cigarette comprising: preparing a blend of cured tobacco, wherein the blend comprises a non-transgenic Burley tobacco and a non-transgenic Flue-cured or Bright tobacco, wherein the non-transgenic

Burley tobacco in the blend is present in an amount of 45-70% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco, and the non-transgenic Flue-cured or Bright tobacco in the blend is present in an amount of 30-55% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco; determining at least one of total pore volume and percentage pore volume distribution of an activated carbon; selecting an activated carbon having a total pore volume of 0.1 mL/g to 0.9 mL/g and/or having a certain percentage of the activated carbon having a pore volume distribution of 0.1 mL/g to 0.9 mL/g, wherein the percentage of carbon having the pore volume distribution is least about 50%; optionally measuring and/or selecting an activated carbon having an average pore diameter of 0.6 nm to 1.1 nm; incorporating the selected activated carbon into a cigarette filter; and generating a filtered cigarette that contains the blend of cured tobacco and the cigarette filter. In the method, the cigarette filter may further comprise a weak base amine-containing resin. The ratio of the activated carbon to the weak base amine-containing resin in the filter in the method may be from about 1:1 to 4:1. The weak base amine-containing resin used in the method may contain at least or equal to 1.3-1.5%, such as 1.4% nitrogen atoms (N) in the form of amine functional groups. In one embodiment, all of the nitrogen atoms are in the form of primary amine functional groups, and in other embodiments the nitrogen atoms are in the form of a mixture that includes primary amine functional groups. The activated carbon used in the method may have an activity of 50-60.

[0021] Another aspect is directed to method of making a filtered cigarette comprising: preparing a blend of cured tobacco, wherein the blend comprises a non-transgenic Burley tobacco and a non-transgenic Flue-cured or Bright tobacco, wherein the non-transgenic Burley tobacco in the blend is present in an amount of 85-92% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco, and the non-transgenic Flue-cured or Bright tobacco in the blend is present in an amount of 8-15% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco; determining at least one of total pore volume and pore volume distribution of an activated carbon; selecting an activated carbon having a total pore volume of 0.5 mL/g, a pore volume distribution of 0.1 mL/g to 0.9 mL/g or a pore diameter of 0.6 nm to 1.1 nm, wherein the

percentage of carbon having the pore volume distribution is least about 50%; optionally selecting an activated carbon having an average pore diameter of 0.6 nm to 1.1 nm; incorporating the selected activated carbon into a cigarette filter; and generating a filtered cigarette that contains the blend of cured tobacco and the cigarette filter. In the method, the cigarette filter may further comprise a weak base amine-containing resin. The ratio of the activated carbon to the weak base amine-containing resin in the filter in the method may be from about 1:1 to 4:1. The weak base amine-containing resin used in the method may contain at least or equal to 1.3-1.5%, such as 1.4% nitrogen atoms (N) in the form of amine functional groups. In one embodiment, all of the nitrogen atoms are in the form of primary amine functional groups, and in other embodiments the nitrogen atoms are in the form of a mixture that includes primary amine functional groups. The activated carbon used in the method may have an activity of 50-60.

[0022] In another embodiment, the method further comprises: generating mainstream smoke from the filtered cigarette; and measuring the presence or absence of a toxicant retained in the filter. In addition, the method may further comprise: generating mainstream smoke from the filtered cigarette; and measuring the appearance of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in lung cells contacted with the mainstream smoke, a fraction of the mainstream smoke, or a smoke condensate.

[0023] Another aspect of the invention is directed to a kit comprising: a first cigarette comprising a first cigarette filter that comprises a carbon or a weak base amine-containing resin, or both; and a second cigarette comprising a second cigarette filter that comprises a carbon, a weak base amine-containing resin, or both, wherein the second cigarette filter is configured to retain a greater amount of a toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the first cigarette filter. In the kit, the second cigarette filter may comprise a greater amount of the carbon or the weak base amine-containing resin or both than the first cigarette filter.

[0024] Another aspect of the invention is directed to a method of reducing biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in cells that contact cigarette smoke comprising: advising a tobacco consumer of

the need to reduce the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in cells that contact cigarette smoke; and replacing a cigarette habitually consumed by the tobacco consumer with any of the cigarettes described above.

[0025] Another aspect of the invention is directed to a method of reducing biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in cells of a tobacco consumer comprising: identifying the tobacco consumer in need of a reduction in biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in cells of the tobacco consumer; and replacing a cigarette habitually consumed by the identified tobacco consumer with any of the cigarettes described above. The identifying step may comprise analyzing the presence of DNA DSBs, cell death or perturbation of RNA transcriptome or proteome in cells of the tobacco consumer. This method may further comprise measuring the presence of DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in the cells of the tobacco consumer before and after providing any of the cigarettes described above. The cells may comprise lung cells, cheek cells, throat cells, or buccal cells.

[0026] Another aspect of the invention is directed to a method of gradually reducing the exposure of a tobacco user to a toxicant that caudrd biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome comprising: identifying the tobacco user to receive a gradual reduction in exposure to a toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells; replacing a cigarette habitually consumed by the identified tobacco user with a first cigarette for a predetermined length of time, wherein the first cigarette comprises a first cigarette filter that comprises a carbon, a weak base amine-containing resin, or both; replacing the first cigarette with a second cigarette after the predetermined length of time, wherein the second cigarette comprises a second cigarette filter that comprises the carbon, the poly-amine containing resin, or both, wherein the second cigarette filter is configured to retain a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the first cigarette filter. The predetermined length of time may be about 3-6 weeks.

[0027] In another embodiment, this method may further comprise replacing the second cigarette after a second predetermined length of time with a third cigarette, wherein the third cigarette comprises a third cigarette filter that comprises the carbon, the weak base amine-containing resin, or both, wherein the third cigarette filter is capable of retaining a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the second cigarette filter.

[0028] Another aspect of the invention is directed to a method of marketing a cigarette that is configured to reduce the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome comprising: replacing a cigarette habitually consumed by a tobacco consumer with a first cigarette comprising a first cigarette filter comprising a carbon and a weak base amine-containing resin for a predetermined length of time; replacing the first cigarette after the predetermined period of time with a second cigarette comprising a second cigarette filter configured to retain a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the first cigarette filter; and marketing the first and second cigarettes, wherein the first cigarette is introduced to a consumer prior to the second cigarette and the first cigarette is marketed for a time sufficient to adjust a tobacco consumer's taste prior to marketing the second cigarette. The time to adjust the tobacco consumer's taste may be less than 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months.

[0029] In another embodiment, the method of marketing the cigarette further comprises replacing the second cigarette with a third cigarette that comprises a third cigarette filter capable of retaining a greater amount of a toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the second cigarette filter. In another embodiment, the first cigarette, the second cigarette and the third cigarette have substantially similar packaging. In further embodiment, the first cigarette, the second cigarette, and the third cigarette are sold under the same brand. In yet another embodiment, the first cigarette, the second cigarette, and the third cigarette have the same packaging.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] Figure 1 depicts a multiple section filter cigarette.

[0031] Figure 2 depicts a chart comparing DSBs for cigarette samples containing 90:10 Flue-cured:Burley tobacco and filters comprising varying amounts of activated carbon (TA95). The DSB level in relative fluorescence units compared to sham control are illustrated for cigarette smoke from cigarette samples having one (1) conventional cellulose acetate (CA) filter and four (4) filters containing 40 mg, 60 mg, 80 mg, and 100 mg of TA95 activated carbon.

[0032] Figure 3 depicts a chart comparing DSBs that were measured using an H2AX assay (discussed below in Example 1 in detail) for cigarette samples containing 10:90 Flue-cured:Burley tobacco and filters comprising varying amounts of activated carbon (TA95). The DSB level in relative fluorescence units compared to sham control are illustrated for cigarette samples having one (1) conventional cellulose acetate (CA) filter, and four (4) filters containing 40 mg, 60 mg, 80 mg, and 100 mg of TA95 activated carbon.

[0033] Figure 4 illustrates a comparison of H2AX damage by the blend type of the cigarette sample. The chart illustrates the amount of DSBs based on an H2AX assay as a percentage of DSBs induced by cigarette smoke from a control cigarette of the same blend type having a cellulose acetate filter. The results are shown for cigarette samples comprising a 90:10 Flue-cured:Burley tobacco blend (left/blue) and a 10:90 Flue-cured:Burley tobacco blend (right/red). The DSB percentages are illustrated for cigarette samples comprising four (4) filters containing 40 mg, 60 mg, 80 mg, and 100 mg of TA95 activated carbon. The results show that the H2AX damage using the 10:90 Flue-cured:Burley tobacco blend is significantly lower than the 90:10 Flue-cured:Burley tobacco blend.

[0034] Figure 5 illustrates a comparison of cloning efficiency by the blend type of the cigarette sample. The percentage of cell death using clonogenic assay (discussed below in Example 1 in detail) is illustrated using a cigarette smoke from a cigarette sample having a cellulose acetate control filter for 90:10 Flue-cured:Burley tobacco blend (left/blue) 10:90 Flue-cured:Burley tobacco blend (right/red). Cell death as a percentage of total cells is illustrated for cigarette samples having one (1) conventional cellulose acetate (CA) filter cigarette of the same blend type as the control and two (2) filters containing 40 mg and 100 mg of TA95 activated carbon. The chart shows that cell death based on a cigarette sample

having the 10:90 Flue-cured:Burley tobacco blend is significantly lower than the 90:10 Flue-cured:Burley tobacco blend in the cigarette sample having a filter with 100 mg of activated carbon (TA95).

[0035] Figure 6 depicts a chart comparing DSBs that were measured using an H2AX assay for cigarette samples containing 50:50 Flue-cured:Burley tobacco. The DSB level in relative fluorescence units compared to sham control are illustrated for cigarette samples containing three (3) filters containing 50 mg TA95 activated carbon, 50 mg A109 ion exchange resin, and 50 mg each of TA95 and A109, in comparison to a control conventional cigarette having a cellulose acetate (CA) filter. The cigarette samples having filters containing 50 mg TA95 activated carbon or 50 mg A109 ion exchange resin did not result in a reduction in the DSBs in comparison to the control; however, the cigarette sample containing a filter combining 50 mg each of TA95 and A109 illustrated an unexpected synergistic effect.

[0036] Figure 7 shows a comparison of the percentage of cell death that was measured using a clonogenic assay for cigarette samples containing 50:50 Flue-cured:Burley tobacco blends. The results compare cigarette samples containing three (3) filters containing 50 mg TA95 activated carbon, 50 mg A109 ion exchange resin, and 50 mg each of TA95 and A109 in comparison to a control having conventional cellulose acetate (CA) filter. The cigarette sample having a filter comprising 50 mg TA95 activated carbon resulted in an approximately 10% decrease in cell death in comparison to the control. The cigarette sample having a filter comprising 50 mg A109 ion exchange resin resulted in a slightly higher percentage of cell death in comparison to the control. The cigarette sample having a filter comprising a combination of 50 mg each of TA95 and A109 resulted in a greater than 40% reduction in cell death in comparison to the control, which is significantly greater than what would be expected from an additive effect.

[0037] Figure 8 illustrates the synergistic reduction of the number of DSBs as measured by H2AX at various efficacies of three cigarettes having filters comprising 50/50 v/v of TA95 and each of three resins A109, Duolite and CR20 compared to a 100 mg TA95 control cigarette of the same tobacco blend. A109 is a weak base primary amine resin containing about 1.4% nitrogen atoms (N) in the form of primary amines, whereas Duolite and CR20 are weak base primary amine resins containing about 1.4% nitrogen atoms (N) but

in a mixed composition of primary, secondary and tertiary amine functional groups. As the percentage of primary amine functional groups increase within the resin, the amount of DSBs is reduced. At the same time, however, increasing the percentage of primary amine functional groups within the resin results in increasingly poor sensory/taste characteristics of the cigarette.

[0038] Figure 9 illustrates the synergistic reduction of the number of DSBs as measured by H2AX of filters containing a mixture of sepiolite and A109 resin in comparison to a filter containing sepiolite alone.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0039] Creating a cigarette that produces the least amount of biological insult while retaining taste is desirable in the cigarette market. Approaches are being developed to analyze biological insult such as described in WO2006/124448, which is expressly incorporated by reference herein in its entirety. In the past, attempts have been made to overcome this problem by using low alkaloid tobacco (WO2006/124448). In addition, others in the field have used filters.

[0040] In the past, cigarettes containing tobacco blends having a large proportion of Burley tobacco also contained a relatively higher amount of precursors that deliver tobacco specific nitrosamines (TSNAs), which are carcinogens, to a tobacco consumer. Cigarettes containing a large proportion of Burley, and having lengthy carbon filters, although effective for reducing the amount of TSNAs, resulted in cigarettes that did not satisfy tobacco users' sensory/perception needs, *i.e.*, such cigarettes had an unacceptable taste. For example, a prototype cigarette was developed by Philip Morris having a lengthy filter measuring approximately 32 mm but the cigarette did not enjoy appreciable sales due to unacceptable taste.

[0041] An illustration of the effect of the amount a carbon on DSBs is shown in Example 1. Specifically, DNA double strand breaks (DSBs) in relative fluorescence units compared to sham control decreased upon increasing amounts (40 mg, 60 mg, 80 mg, or 100 mg) of activated carbon present in the filter as shown in Figures 2-3 for cigarette samples containing about 90:10 Flue-cured:Burley tobacco (Figure 2) or 10:90 Flue-cured:Burley tobacco (Figure 3).

[0042] Surprisingly, however, it was found that cigarettes containing tobacco blends having a lower proportion of non-transgenic Flue-cured or Bright tobacco to non-transgenic Burley tobacco resulted in less biological insult (for example, DSBs, cell death or perturbation of RNA transcriptome or proteome) in comparison to cigarettes containing tobacco blends having a relatively higher proportion of non-transgenic Flue-cured or Bright tobacco to non-transgenic Burley tobacco. It was not expected that a cigarette containing a higher proportion of Burley tobacco, which contains a higher amount of carcinogenic TSNAs than Flue-cured or Bright tobacco, would result in a cigarette that induces relatively less biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to the amount of biological insult induced by a conventional or reference cigarette (e.g., 2R4F). Thus, in one embodiment, a cigarette is configured to produce a mainstream smoke that generates less biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to mainstream smoke from a 2R4F reference cigarette under the same smoking conditions. In one embodiment, the mainstream smoke generates 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% produce a mainstream smoke that generates less biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in cells, such as lung cells, such as fewer DSBs in cells, such as A549 cells in a H2AX assay described herein, than the mainstream smoke from a 2R4F reference cigarette under the same smoking conditions.

[0043] Cigarettes containing tobacco blends having a lower proportion of non-transgenic Flue-cured or Bright tobacco to non-transgenic Burley tobacco result in less biological insult as shown in Example 1. Specifically, H2AX damage by a carbon filtered cigarette sample comprising the 90:10 Flue-cured:Burley tobacco blend (Figure 4, left/blue) was compared to a cigarette sample comprising 10:90 Flue-cured:Burley tobacco blend (Figure 4, right/red), wherein the filters associated with each blend contained increasing amounts, namely 40 mg, 60 mg, 80 mg, or 100 mg, of TA95 activated carbon. Figure 4 illustrates that the H2AX damage using the 10:90 Flue-cured:Burley tobacco blend was significantly lower than the 90:10 Flue-cured:Burley tobacco blend.

[0044] A similar comparison was made using the clonogenic assay described above. Specifically, the percentage of cell death by a carbon filtered cigarette sample

comprising the 90:10 Flue-cured:Burley tobacco blend (Figure 5, left/blue) was compared to a cigarette sample comprising 10:90 Flue-cured:Burley tobacco blend (Figure 5, right/red), wherein the filters associated with each blend contained 40 mg or 100 mg of TA95 activated carbon. Surprisingly, in the cigarette sample having a filter comprising 100 mg of activated carbon (TA95), cell death was significantly lower for the 10:90 Flue-cured:Burley tobacco blend in comparison to the 90:10 Flue-cured:Burley tobacco blend as shown in Figure 5.

[0045] A cigarette filter also plays a role in reducing biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to the amount of biological insult induced by a conventional or reference cigarette (e.g., 2R4F). In general, as the length of a carbon filter increases, the biological insult conferred to a tobacco user decreases, for example, the induction of DSBs, cell death or perturbation of RNA transcriptome or proteome decreases. Surprisingly, it has been found that carbon filters, such as an activated carbon filter, are more effective in reducing H2AX damage in cigarettes containing a higher proportion by weight of Burley tobacco with respect to Flue-cured tobacco, such as shown in Figure 4 described above, in comparison to cigarettes containing a higher proportion by weight of Flue-cured tobacco with respect to Burley tobacco.

[0046] At the same time, as mentioned above, carbon filters that are too lengthy are not well accepted due to unacceptable taste. In general, carbon filters greater than 60 mm have not been well accepted. Thus, in one embodiment, the amount of a carbon in a filter must be present in an amount to fulfill the sensory/perception needs of the consumer. In addition to reduced biological insult, surprisingly, the change in taste of carbon filtered cigarettes containing a higher proportion by weight of Burley tobacco with respect to Flue-cured tobacco is less in comparison to cigarettes containing a higher proportion by weight of Flue-cured tobacco with respect to Burley tobacco.

[0047] Accordingly, in one embodiment, a cigarette contains a blend at least 8% by weight of Flue-cured tobacco (such as Virginia Bright leaf), for example 8%, 9%, 10%, 11%, 12%, 13%, 14%, or 15% by weight based on the combined weight of the non-transgenic Flue-cured tobacco and non-transgenic Burley tobacco. Ranges of Flue-cured tobacco in the blend may include 8-15%, such as any one of 8-9%, 8-10%, 8-11%, 8-12%, 8-13%, 8-14%, 9-10%, 9-11%, 9-12%, 9-13%, 9-14%, 9-15%, 10-11%, 10-12%, 10-13%, 10-

14%, 10-15%, 11-12%, 11-13%, 11-14%, 11-15%, 12-13%, 12-14%, 12-15%, 13-14%, 13-15%, or 14-15% by weight based on the combined weight of the non-transgenic Flue-cured tobacco and non-transgenic Burley tobacco. Therefore, non-transgenic Burley tobacco may be present in the blend in an amount of at least 85%, for example 85%, 86%, 87%, 88%, 89%, 90%, 91%, or 92% by weight based on the combined weight of the non-transgenic Flue-cured tobacco and non-transgenic Burley tobacco. Ranges of Burley tobacco in the blend may include about 85-92%, such as any one of 85-86%, 85-87%, 85-88%, 85-89%, 85-90%, 85-91%, 85-92%, 86-87%, 86-88%, 86-89%, 86-90%, 86-91%, 86-92%, 87-88%, 87-89%, 87-90%, 87-91%, 87-92%, 88-89%, 88-90%, 88-91%, 88-92%, 89-90%, 89-91%, 89-92%, 90-91%, 90-92%, or 91-92%, by weight based on the combined weight of the non-transgenic Flue-cured tobacco and non-transgenic Burley tobacco.

[0048] In another embodiment, a cigarette contains a blend at least 30% by weight of Flue-cured tobacco (such as Virginia Bright leaf), for example 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, or 55% by weight based on the combined weight of the non-transgenic Flue-cured tobacco and non-transgenic Burley tobacco. Ranges of Flue-cured tobacco in the blend may include 30-55%, such as any one of 45-55%, 46-54%, 47-53%, 48-52%, 49-51%, or about 50% by weight based on the combined weight of the non-transgenic Flue-cured tobacco and non-transgenic Burley tobacco. Therefore, non-transgenic Burley tobacco may be present in the blend in an amount of at least 45%, for example 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, or 70% by weight based on the combined weight of the non-transgenic Flue-cured tobacco and non-transgenic Burley tobacco. Ranges of Burley tobacco in the blend may include about 45-70%, such as any one of 45-65%, 45-60%, 45-55%, 50-70%, 50-65%, 50-60%, 50-55%, 55-70%, 55-65%, 55-60%, 60-70% or about 50% by weight based on the combined weight of the non-transgenic Flue-cured tobacco and non-transgenic Burley tobacco.

[0049] Surprisingly, it has also been discovered that a filter comprising both a carbon and a weak base amine-containing resin act synergistically and thus, when used in a cigarette, reduce the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to the amount of biological

insult expected by a filter containing either the carbon or the weak base amine-containing resin alone. DSBs induced by a 50:50 Flue-cured:Burley tobacco cigarette having a filter containing 50 mg each of TA95 and A109 were significantly fewer than filters containing either 50 mg TA95 activated carbon or 50 mg A109 ion exchange resin as shown in Figure 6. A cigarette having a filter containing either 50 mg TA95 activated carbon or 50 mg A109 ion exchange resin had no effect in comparison to a reference 2R4F cigarette. However, the combination of 50 mg TA95 activated carbon and 50 mg A109 ion exchange resin showed significant reduction in DSBs. It is expected that various weak base amine-containing resins similarly decrease H2AX damage, and act synergistically when combined with activated carbon as shown in Figure 6.

[0050] Synergistic effects were also observed based on a percentage of cell death using a clonogenic assay for cigarette samples containing 50:50 Flue-cured:Burley tobacco blends. Filters containing 50 mg each of TA95 and A109 showed significant reduction in percentage of cell death in comparison to filters containing either 50 mg TA95 activated carbon or 50 mg A109 ion exchange resin. For instance, A109 (50 mg) does not improve cloning efficiency by itself, but does when mixed with TA95, illustrating a synergistic effect. It is expected that that various weak base amine-containing resins similarly reduce cell death in clonogenic assays when combined with activated carbon, as shown in Figure 7.

[0051] Thus, another embodiment is directed to a cigarette filter containing a proportion by weight of either a weak base amine-containing resin to a carbon or a carbon to weak base amine-containing resin of individually about 50:50, or 40:60 or 30:70, such as 31:69, 32:68, 33:67, 34:66, 35:65, 36:64, 37:63, 38:62, 39:61, 40:60, 41:59, 42:58, 43:57, 44:56, 45:55, 46:54, 47:53, 48:52, or 49:51. In addition to the weight of the carbon, it is advantageous to describe the total pore volume within the filter regardless of weight of carbon. For instance, a 100 mg of carbon type A with a total pore volume of 0.5 mL/g yields a total pore volume in the filter of 0.05 mL. However the 100 mg of carbon type B with a pore volume distribution of 1.0 mL/g yields a total pore volume in the filter is 0.1 mL. Therefore, a filter containing carbon type B will result in less biological insult, for example, fewer DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to the amount of biological insult induced by a conventional or reference cigarette (e.g., 2R4F) than a filter containing the same weight of carbon type A. This result is non-obvious because

pore volumes do not necessarily track with surface area measurements, which is how "high activity" carbons were traditionally selected for removal efficiency. In one embodiment, as less carbon by weight is used in the filter, there is less of an impact on a tobacco consumer's sensory perception and therefore results in a better tasting cigarette.

[0052] In another embodiment, a cigarette filter contains a carbon having a total pore volume of 0.1 mL/g to 0.9 mL/g or about 0.5 mL/g, a pore volume distribution of 0.1 mL/g to 0.9 mL/g, or a pore diameter of 0.6 nm to 1.1 nm, wherein the percentage of carbon having the pore volume distribution is least about 50%, and wherein a carbon is selected for a filter based on a total pore volume, total pore volume distribution and/or pore diameter. Total pore volume of the carbon has significantly more effect on removal of a toxicant from mainstream smoke and reduction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to the amount of biological insult induced by a conventional or reference cigarette (e.g., 2R4F), than the surface area of the carbon. Surprisingly, a carbon having an increased pore volume but the same surface area as a carbon with a lower pore volume will allow increased removal efficiency of vapor phase smoke constituents. The lowering of vapor phase components in smoke leads to a reduction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to the amount of biological insult induced by a conventional or reference cigarette (e.g., 2R4F), resulting in comparatively reduced biological effects. This approach advantageously allows cigarette designs to provide higher reductions in unwanted smoke components by using lower quantities of carbon in the cigarette filter. For example, 50% by weight less of a carbon with the total pore volume distribution described above can be used in comparison to a carbon with half of the total pore volume distribution. In another embodiment, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, less carbon by weight may be used in comparison to a carbon having a total pore volume of less than 0.1 mL/g to 0.9 mL/g. In addition, the same amount of a carbon by weight having the total pore volume distribution described above, will have 75 – 100% more activity (adsorption) than a carbon having half of the total pore volume distribution. In another embodiment, a carbon having the total pore volume distribution described above may have 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, more activity based on

the same amount of a carbon by weight. The use of lower quantities provides a cigarette with increased sensory perception and more consumer acceptability.

[0053] The toxicants reduced in the cigarettes described herein include any of those known to be present in cigarette smoke, including but not limited to, nicotine, tar, and carbon monoxide, as well as formaldehyde, ammonia, sulfur-containing compounds, and hydrogen cyanide. The reduced risk cigarettes described herein may comprise in one embodiment, three components. The first component is a cut filler composition that comprises a cured tobacco blend having at least one tobacco, such as, for example, a non-transgenic Burley tobacco and/or low alkaloid Burley tobacco. In one embodiment, the Burley tobacco has been cured to have a low or reduced level of compounds that can cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to the amount of biological insult induced by a conventional or reference cigarette (e.g., 2R4F), e.g., in human cells. In some embodiments, the cut filler composition comprises a cured tobacco blend having a Burley or a low alkaloid Burley tobacco that has been cured to have a low level of tobacco-specific nitrosamines (TSNAs). The second component is a cigarette wrapper with reduced ignition propensity that circumscribes the cut filler composition. The third component is a cigarette filter that comprises carbon and/or an ion-exchange resin.

[0054] Biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome are generated by a variety of genotoxic agents, and are among the most critical lesions that may lead to apoptosis, mutations, translocations or the loss of significant sections of chromosomal material. Detection of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome upon cell exposure to a potential carcinogen, therefore, provides a way to assess the potential hazard of the exposure to cigarette smoke. In one embodiment, a sensitive assay of DSBs detection based on analysis of histone H2AX phosphorylation is used. Histone H2AX, a variant of a family of at least eight protein species of the nucleosome core histone H2A, becomes phosphorylated in live cells upon induction of DSBs. The phosphorylation of H2AX on Ser 139 at sites flanking the DSBs is carried out by ATM-, ATR-, and/or DNA-dependent protein kinases (DNA-PKs). The phosphorylated form of H2AX is denoted γH2AX. Antibodies and fragments thereof, and related methods for selectively detecting gamma-H2AX, are known in

the art, as exemplified in U.S. Pat. Nos. 6,362,317 and 6,884,873, both of which are hereby expressly incorporated by reference in their entireties. This and additional methods for assessing the level of compounds that can cause DSBs in human cells are known in the art as exemplified in WO2006/124448 and WO 2005/113821, both of which are hereby expressly incorporated by reference in their entireties.

[0055] In other embodiments, a clonogenic assay or an assay that detects perturbation of the RNA transcriptome or proteome are used, which are described below in more detail.

[0056] Low alkaloid tobacco, for example, tobacco that has been chemically treated or extracted to remove nicotine, or tobacco that has been selectively bred to have low alkaloid or low nicotine levels, such as tobacco having one or more mutations in genes encoding enzymes involved in nicotine biosynthesis, can be employed in the cigarettes described herein, and the inclusion of these reduced risk tobaccos with a filter comprising an ion-exchange resin and/or carbon, results in a cigarette that reduces the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to the amount of induction of biological insult induced by a conventional or reference cigarette (e.g., 2R4F), in cells of a tobacco consumer or in cells contacted with smoke from said cigarettes in an *in vitro* assay, as described herein.

[0057] The level or amount of DNA DSBs induced by the cigarettes described herein may be determined by several types of DNA DSB assays, including the one described in Example I. In certain embodiments, the reduced risk cigarettes described herein induce less than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% fewer DNA DSBs than a conventional or reference cigarette (e.g., 2R4F). Similarly, the cigarettes described herein reduce cell death or perturbation of RNA transcriptome or proteome of less than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% the amount of cell death or perturbation of RNA transcriptome or proteome as compared to a conventional or reference cigarette (e.g., 2R4F).

Reduced Risk Tobacco

[0058] Several approaches to create a reduced risk tobacco product having a reduced amount of a harmful compound are described. At least some of the reduced risk tobacco products provided herein contain modified tobacco. As used herein, "modified tobacco" refers to a tobacco that contains a mutation in a gene involved in nicotine

biosynthesis or has been subjected to one or more genetic modifications (e.g., genetic engineering), chemical or processing steps (e.g., extraction) that is different than the conventional treatment or processing of traditional and/or "wild-type" tobaccos. In one example, a tobacco can be selectively bred for reduced risk properties such as crossing a wild-type tobacco with a tobacco that has a mutation in a gene involved in nicotine biosynthesis (e.g., LA Burley 21 or LA Flue 53). In another example, a tobacco can be chemically modified, by, for example, extracting or chemically altering one or more components of tobacco, according to methods known in the art, as exemplified in U.S. Pat. Nos. 6,789,548, 4,557,280; 4,561,452; 4,848,373; 4,183,364; 4,215,706; 4,257,430; 4,248,251; 4,235,251; 4,216,784; 4,177,822.; 4,055,191 (all of which are herein expressly incorporated by reference in their entireties) or by adding one or more compounds (e.g., an antioxidant) to a tobacco or tobacco plant prior to harvesting the tobacco, as known in the art and exemplified in U.S. Pat. Pub. No. 2005/0072047, herein expressly incorporated by reference in its entirety. Additional modified tobaccos contemplated herein include reconstituted tobacco, extracted tobacco, and expanded or puffed tobacco. Any one or more of these techniques can be combined with the modified tobaccos above and/or as otherwise described herein. In some embodiments, the tobacco is modified to have a reduced amount of a compound that contributes to a tobacco-related disease, including, but not limited to, a compound associated with a tobacco-related disease or a metabolite thereof (*e.g.,* tobacco sterols, nicotine, a TSNA, and a gene product that is involved in the production of a compound associated with a tobacco-related disease or a metabolite thereof). Numerous methods for preparing a reduced-risk tobacco product are known in the art, including, for example, those described in WO2006/124448 and US2006/0157072, which are herein expressly incorporated by reference.

[0059] Researchers have developed several approaches to reduce some of these harmful compounds, but many of these conventional techniques result in a product that has poor taste, fragrance, or smoking properties. Some processes, for example, reduce the nicotine content of tobacco by selective breeding, microbial enzymatic degradation, chemical extraction, or high pressure extraction. (*See* e.g., U.S. Pat. Nos. 4,557,280; 4,561,452; 4,848,373; 4,183,364; and 4,215,706, all of which are hereby expressly incorporated by reference in their entireties). More recently, techniques in genetic engineering and

chemically-induced gene suppression have been employed to make reduced nicotine and/or reduced tobacco specific nitrosamine (TSNA) tobacco. (*See e.g.,* Conkling et al., W098/56923; U.S. Pat. Nos.: 6,586,661; 6,423,520; and U.S. Pat. App. Nos. 09/963,340; 10/356,076; 09/941,042; 10/363,069; 10/729,121; 10/943,346; Timko et al., WO 00/67558, which designated the United States and was published in English, Nakatani et al., U.S. Pat. Nos.: 5,684,241; 5,369,023; 5,260,205; and Roberts et al., U.S. Pat. No. 6,700,040, all of which are hereby expressly incorporated by reference in their entireties. Any one or more of these techniques can be used to create a tobacco or tobacco product used with the teachings herein.

[0060] Also provided herein are modified tobaccos and tobacco blends that have reduced levels of compounds that induce biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to the biological insult induced by a control or reference cigarette such as 2R4F, in human cells contacted by smoke from said tobacco, while still maintaining sufficiently desirable consumer taste, so as to receive market acceptance. Methods for assessing sufficiently desirable consumer taste of a tobacco product are well-established in the art and are exemplified elsewhere herein, and include, but are not limited to, limited geographic introduction into the market and/or focus group testing, where the results of such assessments are evaluated according to well-established standards in the art. These methods are employed to develop a step-wise program for introduction of cigarettes that gradually (in a step-wise fashion) reduce the levels or amounts of a toxicant in cigarette smoke while maintaining or adjusting consumer sensory/perception of taste or consumer acceptance over time.

[0061] Tobacco products that comprise a modified tobacco described herein include "full-flavor," "lights," and "ultra light" cigarettes typically having a reduced level of compounds that induce biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to the amount of biological insult induced by a conventional or reference cigarette (e.g., 2R4F), in human cells contacted by smoke from said Burley tobacco. The term "tobacco products" includes, but is not limited to, smoking materials (e.g., cigarettes, cigars, pipe tobacco), snuff, snus, chewing tobacco, gum, and lozenges. The term "reduced risk tobacco product" or "reduced risk tobacco" includes, but is not limited to, a tobacco product or tobacco comprising a modified tobacco that has a

reduced amount of a compound that contributes to a tobacco-related disease, such as nicotine, nornicotine, a sterol, or the metabolites thereof including, but not limited to, a TSNA, or harmful compounds generated upon pyrolysis of tobacco, including but not limited to, acrolein, aldehydes, aromatic amines, aromatic hydrocarbons, carbon monoxide, carbonyl compounds, free radicals, hydrogen cyanide, nitriles, oxides of nitrogen, phenolics, polycyclic aromatic hydrocarbons, volatile hydrocarbons, as compared to the amount of these compounds in or generated by a reference tobacco or reference tobacco product (e.g., IM16, 2R4F or 1R5F), a commercially available tobacco product of the same class (e.g., full-flavor, lights, and ultra-lights), or a tobacco of the same variety (e.g., Burley, Virginia Flue-cured, or Oriental) or strain (e.g., LA Burley 21, K326, Tn90, Djebel174) as the modified tobacco). In some embodiments, the "reduced risk tobacco product" or "reduced risk tobacco" is a tobacco product or tobacco that contains an additive that reduces the harmful effects of conventional tobacco (e.g., reduces the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to the amount of biological insult induced by a conventional tobacco.

[0062] Various tobaccos may be used in accordance with the embodiments described herein. For example, MB1 is a higher than normal Burley blend. In addition, MB2 is a low nitrosamine Burley.

[0063] The modified tobacco described herein is suitable for conventional growing and harvesting techniques (e.g. topping or no topping, bagging the flowers or not bagging the flowers, cultivation in manure rich soil or without manure) and the harvested leaves and stems are suitable for use in one or more traditional tobacco products including, but not limited to, pipe, cigar and cigarette tobacco and chewing tobacco in any form including leaf tobacco, shredded tobacco or cut tobacco. It is also contemplated that the modified tobacco described herein can be processed and blended with conventional tobacco so as to create a wide range of tobacco products with varying amounts of compounds that are harmful in the tobacco or smoke generated therefrom.

[0064] In some embodiments, the reduced risk modified tobacco is a selectively bred low alkaloid tobacco, such as, for example, LA Burley 21, LA Flue 53, and other known low alkaloid tobacco strains. This tobacco can be blended with conventional tobacco to yield

a blend of tobacco with varying taste characteristics and/or toxicants in the tobacco or smoke generated therefrom.

[0065] In some embodiments, the reduced risk modified tobacco is a tobacco grown under altered conditions that result in a reduced amount of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to the amount of biological insult induced by a conventional or reference cigarette (e.g., 2R4F) in human cells, nicotine and TSNA. Exemplary conditions include, but are not limited to reduced nitrate soils, increased watering of tobacco plants, use of clay soils in preference to volcanic soils, and any other conditions known in the art for reducing the amount of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells, nicotine, TSNA, sterol and/or PAH in tobacco. The "reduced risk" modified tobacco described herein can also be processed or cured or otherwise treated so as to reduce the amount of a harmful compound (e.g., aseptic processing, removal of microbes, bacteria or fungi or mold, air curing, stalk cutting wherein the tobacco is not contacted with the soil, and aseptic packaging).

[0066] In some embodiments, the modified tobacco has reduced levels of nicotine and/or nornicotine. Alkaloids such as nicotine and nornicotine are precursors for a number of harmful compounds that contribute to tobacco-related disease (e.g., the tobacco specific nitrosamines (TSNAs): N'-nitrosonornicotine (NNN), N'-nitrosoanatabine (NAT), N'-nitrosoanabasine (NAB), 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), 4-(N-nitrosomethylamino)-4-(3-pyridyl)-1-butanal (NNA)-4-N-nitrosomethylamino)-1-(3-pyridyl)-1-butanol (NNAL), 4-N-nitrosomethylamino)-4-(3-pyridyl)-1-butanol (iso-NNAL) and/or 4-(N-nitrosomethylamino)-4-(3-pyridyl)-butanoic acid (iso-NNAC)). Sterols are precursors for a number of harmful compounds, which are generated by pyrolysis of tobacco, that also contribute to tobacco-related disease (e.g., polycyclic aromatic hydrocarbons (PAHs), such as benz[a]pyrene (BAP), heterocyclic hydrocarbons, terpenes, paraffins, aromatic amines, and aldehydes). Because the presence of these harmful compounds in tobacco contributes to tobacco-related disease, a modified tobacco that comprises a reduced amount of any one of these compounds, as compared to a reference tobacco (e.g., the industry standard reference tobacco IM16 (Philip Morris® USA) or the low tar reference

cigarette 2R4F or the ultra low tar cigarette 1R5F, which are Kentucky reference cigarettes that can be obtained from the Tobacco and Health Institute at the University of Kentucky, a conventional tobacco (e.g., a commercially available tobacco of the same class (e.g., "full-flavor" or "light" or "ultra-light") or a non-transgenic tobacco (e.g., a tobacco of the same variety, such as Burley, Virginia Flue-cured, or Oriental, can be assessed, for example, by measuring a reduced level of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells contacted by smoke from the reduced risk tobacco. Tobacco products comprising the modified tobacco can also be analyzed by various approaches to confirm that the tobacco is "reduced risk," as compared to a parental strain or a reference tobacco using a H2AX analysis, clonogenic analysis or RNA transcriptome or proteome analysis.

[0067] The modified tobacco can be sterilized or otherwise made substantially free of microbes, and said tobacco can be incorporated into tobacco products, preferably, cigarettes, optionally, by an aseptic approach so as to not introduce microbes (e.g., bacteria, mold, yeast, and fungi) into the products. Tobacco products comprising the modified tobacco can then be packaged, optionally, by an aseptic approach in air-tight or microbe-free yeast, mold and fungi-free packaging so as to not introduce microbes yeast, mold or fungi into the products. In this manner, the conversion of alkaloid to TSNA, which results from microbial growth on the tobacco when microbes are introduced during processing, packaging, and storage, is significantly reduced. By using the embodied tobacco preparative methods, which may include several aseptic processing, manufacturing, and packaging procedures, one can maintain an amount of total TSNA (e.g., the collective content of NNN, NAT, NAB, and NNK) in or delivered by (e.g., as measured by FTC or ISO methodologies) a commercially available tobacco product of less than or equal to 0.5μg/g (e.g., 0.05μg/g, 0.1μg, 0.2μg/g, 0.3μg/g, 0.4μg/g, or 0.5μg/g) for a period of at least 1 week, 1 month, or 1-5 years after packaging or incorporation of the tobacco into a tobacco product (e.g., at least 1-30 days, 30 – 90 days, 90-180 days, 180-270 days, 270 days - 365 days, 1 year - 1.5 years, 1.5 – 2.0 years, 2.0 years – 2.5 years, 2.5 years – 3.0 years, 3.0 years – 4 years, and 4.0 years – 5.0 years).

[0068] The reduced risk modified tobacco can be made substantially free of microbes (e.g., an aseptic preparation) by employing sterilization, heat treatment,

pasteurization, steam treatment, gas treatment, and radiation (e.g., gamma, microwave, and ultraviolet). The term "substantially free of microbes" in some contexts can mean an amount of bacteria, mold, fungi, or yeast that is reduced to the point that the conversion of nicotine or total alkaloid to TSNA is negligible (e.g., the resultant concentration of or the amount of delivered or provided total TSNA (e.g., NNN, NNK, NAT, and NAB) in or delivered by a tobacco or tobacco product is equal to or below 0.5μg/g (e.g., 0.05μg/g, 0.1μg, 0.2μg/g, 0.3μg/g, 0.4μg/g, or 0.5μg/g) after prolonged storage (e.g., at least 1-30 days, 30 – 90 days, 90-180 days, 180-270 days, 270 days - 365 days, 1 year - 1.5 years, 1.5 – 2.0 years, 2.0 years – 2.5 years, 2.5 years – 3.0 years, 3.0 years – 4 years, and 4.0 years – 5.0 years)). The term "substantially free of microbes" also includes the term "substantially free of bacteria," which means in some contexts that the tobacco or tobacco product is substantially free of *Arthrobacter, Proteus,* nicotine oxidizing bacteria, such as P-34, *Psuedomonas, Xantomonas, or Zoogloea* strains of bacteria. For example, a tobacco or tobacco product is substantially free of bacteria or a particular strain of bacteria when said tobacco or tobacco product has less than or equal to 20% of the bacteria or a specific strain of bacteria normally present on the tobacco or tobacco product in the absence of application of a technique to rid the tobacco or tobacco product of bacteria (e.g., less than or equal to 1%, 2%, 3%, 4% 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20%). With respect to modified tobacco described herein, the term "substantially free of bacteria" can refer to tobacco or a tobacco product containing the modified tobacco that has less than or equal to 20% of the bacteria normally present on the strain of tobacco prior to modification and/or application of a technique to rid the tobacco or tobacco product of bacteria (e.g., less than or equal to 1%, 2%, 3%, 4% 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20%).

[0069] In some embodiments, a modified tobacco comprises a reduced amount of alkaloid (e.g., a reduced amount of nicotine, nornicotine, and/or TSNAs), which can then be contacted with an exogenous nicotine so as to raise the level of nicotine in the contacted modified tobacco in a controlled fashion. By this approach, nicotine levels in modified tobacco that comprises a reduced amount of endogenous nicotine can be selectively raised to levels that are commensurate with conventional full-flavor cigarettes, light cigarettes, or ultra-light cigarettes. (*See e.g.,* WO 2005/018307, herein expressly incorporated by reference

in its entirety.) For example, modified tobacco comprising a reduced amount of endogenous nicotine and/or TSNAs can be contacted with an amount of exogenous nicotine that is at least, equal to, or more than 0.3 mg/g – 20.0mg/g (nicotine/gram of tobacco). In some of the aforementioned embodiments, the modified tobacco contacted with the exogenous nicotine is a tobacco from a plant that has one or more mutations in a gene involved in nicotine biosynthesis (e.g., LA Burley 21 or LA Flue 53).

[0070] It should also be appreciated that in some embodiments, the reduced risk tobacco products comprise an amount of tar similar to the amount of tar in standard cigarettes. While not intending to be limited to the following, it has been postulated that consumer acceptance of a cigarette is related to the amount of tar in the product, and, accordingly, by delivering a similar amount of tar but fewer toxicants in the smoke in a reduced risk tobacco product, the product will likely be accepted by a consumer. In such embodiments, the tobacco product (e.g. a cigarette) can have about 0.5 mg to about 30 mg of tar. Such a tobacco product comprises (e.g., on the leaf or tobacco rod) or delivers (e.g., side-stream or main-stream smoke by the FTC and/or ISO methods), for example, less than or equal to 0.5 mg, 1 mg, 1.5 mg, 2 mg, 2.5 mg, 3 mg, 3.5 mg, 4 mg, 4.5 mg, 5 mg, 5.5 mg, 6 mg, 6.5 mg, 7 mg, 7.5 mg, 8 mg, 8.5mg, 9 mg, 9.5 mg, 10 mg, 10.5 mg, 11 mg, 11.5 mg, 12 mg, 12.5 mg, 13 mg, 13.5 mg, 14 mg, 14.5 mg, 15 mg, 15.5 mg, 16 mg, 16.5 m, 17 mg, 17.5 mg, 18 mg, 18.5 mg, 19 mg, 19.5 mg, 20 mg, 20.5 mg, 21 mg, 21.5 mg, 22 mg, 22.5 mg, 23 mg, 23.5 mg, 24 mg, 24.5 mg, 25 mg, 25.5 mg, 26 mg, 26.5 mg, 27 mg, 27.5 mg, 28 mg, 28.5 mg, 29 mg, 29.5 mg, or 30 mg of tar.

Reducing the amount of nicotine, TSNAs and sterols in tobacco

[0071] As discussed above, TSNAs, nicotine, nornicotine, and sterols contribute significantly to tobacco-related disease, most notably the carcinogenic potential of tobacco and tobacco products. Thus, tobacco and tobacco products that have or produce reduced amounts of these compounds are reduced risk compositions (e.g., products that have a reduced potential to contribute to a tobacco-related disease). Without wishing to be bound by any particular theory, it is contemplated that the creation of tobacco plants, tobacco and tobacco products that have a reduced amount of nicotine and/or related alkaloids can also have reduced amounts of TSNAs. That is, by removing or reducing nicotine in tobacco plants, tobacco and tobacco products, one effectively removes the most significant alkaloid

substrate for TSNA formation. TSNAs can also be reduced by employing modified harvesting (e.g., stalk cutting wherein the tobacco is not contacted with the soil), as well as air curing and growing in steps to avoid an accumulation of nitrates (e.g., growing in clay soils with low nitrogen) and steps to avoid introduction of microbes (e.g., sterilization of green tobacco). Similarly, it is contemplated that by reducing sterols in tobacco blends, one can reduce the amount of PAHs generated from pyrolysis of the tobacco (e.g., using selectively bred tobacco having a reduced level of sterols).

[0072] It should be emphasized that the phrase "a reduced amount" as applied herein can refer to an amount of a compound in a modified tobacco or a tobacco product that is less than what would be found in a tobacco or a tobacco product from the same variety of tobacco, processed in the same manner, which has not been treated or otherwise provided according to the methods provided herein, or can refer to an amount of a compound in a modified tobacco or a tobacco product that is less than what would be found in a reference tobacco or tobacco product. Thus, in some contexts, wild-type tobacco of the same variety that has been processed in the same manner is used as a control by which to measure whether a reduction in a particular compound is present.

[0073] Some embodiments comprise cured tobaccos (e.g., Burley, Flue-cured, or Oriental) with reduced amounts of nicotine as compared to control varieties, wherein the amount of nicotine delivered by the product (e.g., as measured by FTC or ISO methodologies) is less than about 2mg nicotine in smoke/g tobacco, 1mg/g, 0.75mg/g, 0.5 mg/g or desirably less than about 0.1 mg/g, and preferably less than 0.08mg/g, 0.07mg/g, 0.06mg/g, 0.05mg/g, 0.04mg/g, 0.03mg/g, 0.02mg/g, 0.01mg/g. Tobacco products made from these reduced nicotine and TSNA tobaccos are also embodiments. In some embodiments, the cigarettes comprise a low alkaloid tobacco (e.g., LA Burley 21 or LA Flue 53), wherein the amount of nicotine in the tobacco (e.g., the tobacco blend) is from about 0.2 mg/g to about 30 mg/g. In some embodiments, the cigarettes comprise a low alkaloid tobacco (e.g., LA Burley 21 or LA Flue 53), wherein the amount of nicotine in the tobacco (e.g., the tobacco blend) is from about 0.5 mg/g to about 20 mg/g. In some embodiments, the cigarettes comprise a low alkaloid tobacco (e.g., LA Burley 21 or LA Flue 53), wherein the amount of nicotine in the tobacco (e.g., the tobacco blend) is from about 1.0 mg/g to about 15 mg/g.

[0074] Alternatively, a cigarette provided herein can have or deliver, for example, less than or equal to 0.1mg, 0.15mg, 0.2mg, 0.25mg, 0.3mg, 0.35mg, 0.4mg, 0.45mg, 0.5mg, 0.55mg, 0.6mg, 0.65mg, 0.7mg, 0.75mg, 0.8mg, 0.85mg, 0.9mg, 0.95mg, 1.0mg, 1.1mg, 1.15mg, 1.2mg, 1.25mg, 1.3mg, 1.35mg, 1.4mg, 1.45mg, 1.5mg, 1.55mg, 1.6mg, 1.65mg, 1.7mg, 1.75mg, 1.8mg, 1.85mg, 1.9mg, 1.95mg, 2.0mg, 2.1mg, 2.15mg, 2.2mg, 2.25mg, 2.3mg, 2.35mg, 2.4mg, 2.45mg, 2.5mg, 2.55mg, 2.6mg, 2.65mg, 2.7mg, 2.75mg, 2.8mg, 2.85mg, 2.9mg, 2.95mg, 3.0mg, 3.1mg, 3.15mg, 3.2mg, 3.25mg, 3.3mg, 3.35mg, 3.4mg, 3.45mg, 3.5mg, 3.55mg, 3.6mg, 3.65mg, 3.7mg, 3.75mg, 3.8mg, 3.85mg, 3.9mg, 3.95mg, 4.0mg,. 4.1mg, 4.15mg, 4.2mg, 4.25mg, 4.3mg, 4.35mg, 4.4mg, 4.45mg, 4.4mg, 4.45mg, 4.5mg, 4.55mg, 4.6mg, 4.65mg, 4.7mg, 4.75mg, 4.8mg, 4.85mg, 4.9mg, 4.95mg, 5.0mg, 5.5mg, 5.7mg, 6.0mg, 6.5mgmg, 6.7mg, 7.0mg, 7.5mg, 7.7mg, 8.0mg, 8.5mg, 8.7mg, 9.0mg, 9.5mg, 9.7mg, 10.0mg, 10.5mg, 10.7mg, and 11.0mg nicotine and less than or equal to 0.001μg, 0.002μg, 0.003μg, 0.004μg, 0.005μg, 0.006μg, 0.007μg, 0.008μg, 0.009μg, 0.01μg, 0.02μg, 0.03μg, 0.04μg, 0.05μg, 0.06μg, 0.07μg, 0.08μg, 0.09μg, 0.1μg, 0.15μg, 0.2μg, 0.25μg, 0.3μg, 0.336μg, 0.339μg, 0.345μg, 0.35μg, 0.375μg, 0.4μg, 0.414μg, 0.45μg, 0.5μg, 0.515μg, 0.55μg, 0.555μg, 0.56μg, 0.578μg, 0.58μg, 0.6μg, 0.611μg, 0.624μg, 0.65μg, 0.7μg, 0.75μg, 0.8μg, 0.85μg, 0.9μg, 0.95μg, 1.0μg, 1.1μg, 1.114μg, 1.15μg, 1.2μg, 1.25μg, 1.3μg, 1.35μg, 1.4μg, 1.45μg, 1.5μg, 1.55μg, 1.6μg, 1.65μg, 1.7μg, 1.75μg, 1.8μg, 1.85μg, 1.9μg, 1.95μg, 2.0μg, 2.1μg, 2.15μg, 2.2μg TSNA (e.g., collective content of NNN, NAT, NAB, and NNK) per cigarette.

Curing

[0075] The curing process brings out the flavor and aroma of tobacco. Several methods for curing tobacco may be used, and indeed many methods have been previously disclosed. For example, U.S. Pat. Nos. 4,499,911 to Johnson; 5,685,710 to Martinez Sagrera; 3,905,123 to Fowler; 3,840,025 to Fowler; and 4,192,323 to Horne (all of which are hereby expressly incorporated by reference in their entireties) describe aspects of the tobacco curing process, which may be used for some embodiments provided herein. Conventionally, "sticks" that are loaded with tobacco are placed into bulk containers and placed into structures known as a curing barn. A Flue can be used in some embodiments (thus earning the term "Flue-cured"). The method of curing will depend, in some cases, on the type of tobacco product desired, (i.e., snuff, snus, cigarettes, or pipe tobacco may preferably utilize

different curing methods) and preferred methods may vary from region to region and in different countries. In some approaches, the stems and midveins of the leaf, which contain a high proportion of TSNAs, are removed from the leaves prior to curing to yield a high quality, low TSNA tobacco product.

[0076] "Flue-curing" is a popular method for curing tobacco in Virginia, North Carolina, and the Coastal Plains regions of the United States. Flue-curing requires a closed building equipped with a system of ventilation and a source of heat. The heating can be direct or indirect (e.g., radiant heat). When heat and humidity are controlled, leaf color changes, moisture is quickly removed, and the leaf and stems dry. Careful monitoring of the heating and humidity can reduce the accumulation of TSNAs.

[0077] Another curing method is termed "air-curing." In this method, an open framework is prepared in which sticks of leaves (or whole plants) are hung so as to be protected from both wind and sun. Leaf color changes from green to yellow, as leaves and stems dry. As contemplated herein, in some embodiments, the air curing of tobaccos such as Burley is performed in an open structure with wide spacing in a manner that ensures exposure of the tobacco to large amounts of air during the curing process so as to obtain a very low TSNA tobacco. Air-curing experiments at a higher temperature have shown that considerably higher levels of N-nitrosamines are formed at a curing temperature of 32°C than at 16°C, which is associated with a rise of the nitrite level in the tobacco, and may also be associated with a rise in microbial enzymatic activity. Modified curing that involves faster drying from wider spacing or from more open curing structures has been shown to reduce TSNA levels in Burley tobacco. The climatic conditions prevailing during curing exert a major influence on N-nitrosamine formation, and the relative humidity during air-curing can be of importance. Stalk curing results in higher TSNA levels in the smoke than primed-leaf curing. Sun-cured Oriental tobaccos have lower TSNA levels than Flue- and air-cured dark tobaccos. Accelerated curing of crude tobaccos, such as homogenized leaf curing, limits the ability of bacteria to carry out the nitrosation reactions. However, many of the methods described above for reducing TSNAs in Burley tobacco can have undesirable effects on tobacco taste.

[0078] TSNA formation in Flue-cured tobacco also results from exposure of the tobacco to combustion gases during curing, where nearly all of the TSNAs in Flue-cured

tobacco (e.g., Virginia Flue-cured) result from a reaction involving NOx and nicotine or other alkaloids. The predominant source of NOx is the mixture of combustion gases in direct-fired barns. At present, Flue-cured tobacco is predominantly cured in commercial bulk barns. As a result of energy pressures in the U.S. during the 1960's, farmer-built "stick barns" with heat- exchanged Flue systems were gradually replaced with more energy efficient bulk barns using direct-fired liquid propane gas (LPG) burners. These LPG direct-fired burner systems exhaust combustion gases and combustion by-products directly into the barn where contact is made with the curing tobacco. Studies indicate that LPG combustion by-products react with naturally occurring tobacco alkaloids to form TSNAs.

[0079] In contrast to direct-fired curing, heat-exchange burner configurations completely vent combustion gases and combustion by-products to the external atmosphere rather than into the barn. The heat-exchange process precludes exposure of the tobacco to LPG combustion by-products, thereby eliminating an important source of nitrosating agent for TSNA formation, without degrading leaf quality or smoking quality. The use of heat exchangers can reduce TSNA levels by about 90%, depending on the type of tobacco.

[0080] "Fire-curing" employs an enclosed barn similar to that used for Flue-curing. The tobacco is hung over low temperature fire so that the leaves cure in a smoke-laden atmosphere. This process uses lower temperatures, so the process may take up to a month, in contrast to Flue-curing, which takes about 6 to 8 days.

[0081] A further curing method, termed "sun-curing" is the drying of uncovered sticks or strings of tobacco leaves in the sun. The best known sun-cured tobaccos are the so-called Oriental tobaccos of Turkey, Greece, Yugoslavia, and nearby countries.

[0082] Although many of the approaches described in this section have significant drawbacks, it should be understood that any or all of these techniques can be used with other techniques, as described herein, to make tobacco and tobacco products having reduced TSNAs.

[0083] The cured tobacco may then be blended with other tobaccos or other materials to create the product to be used herein.

Additional tobacco modifications

[0084] Additional techniques can be used to make the tobacco products provided herein, including but not limited to, chemical modification, expansion, extraction, or puffing, and reconstitution.

[0085] Any of a variety of chemically modified tobaccos can be included in the methods and tobacco products provided herein. For example, the chemical modification can include palladium, or can include an auxin or auxin analog (*see e.g.,* U.S. Pat. No. 6,789,548 and U.S. Pat. App. Pub. No. 20050072047, both of which are hereby expressly incorporated by reference in their entireties).

[0086] By one approach, a chemically modified tobacco is made as follows. A tobacco is provided and a casing solution is applied thereto. Thereafter, a plurality of metallic or carbonaceous catalytic particles having a mean average or a mode average particle size of less than about 20 microns is applied to the tobacco in a form separate from the casing solution. Next, a nitrate or nitrite source in a form separate from the casing solution and in a form separate from the plurality of metallic or carbonaceous catalytic particles is applied to the tobacco, before, after or simultaneously with applying the plurality of particles but after applying the casing solution, whereby a smoking composition is obtained. In some embodiments of this modified tobacco, a polyaromatic hydrocarbon, azaarene, carbazole, or a phenolic compound is reduced.

[0087] In another example, the chemically modified tobacco can be extracted tobacco. By some approaches the chemically modified tobacco is extracted with an organic solvent and other processes use super-critical fluid extraction or carbon dioxide. In another example, the chemical modification can be a biotic modification. Microbes that ingest nitrates and alkaloids can be applied to tobacco so as to obtain a reduced nicotine tobacco; for example such a biotic modification can include bacteria. In another example, the tobacco is processed to remove the presence of a microbe. In another example the chemically modified tobacco can be sterilized, pasteurized, or irradiated.

[0088] Tobacco can also be modified to decrease one or more toxicants such as metabolites, nicotine-related compounds and sterols in smoke generated therefrom. In some methods, a tobacco that has been modified to produce lower levels of one or more toxicants, such as nicotine or a nicotine metabolite, or a sterol, can have exogenously added thereto, an

antioxidant or radical scavenger, for example. Tobacco at any stage of its processing can have added thereto an antioxidant compound or a composition with radical scavenger properties. Any of a variety of known antioxidant compounds and additives containing antioxidants or radical scavengers can be added to the tobacco, including, but not limited to, lycopene, tocopherol, tocopherol metabolites, ascorbic acid, unsaturated fatty acids, N-acetyl cysteine, and other antioxidants known in the art. An additive with antioxidant properties can include a biological composition or extract that can neutralize oxidants, such as milk or milk proteins, turmeric or turmeric extracts, barley or barley extracts, alfalfa or alfalfa extracts. Other compounds that can be added to the tobacco include thiol-containing proteins, plant extracts, aromatic compounds (e.g., caffeine or pentoxyfyllen, which are contemplated to scavenge radicals).

[0089] Another form of modified tobacco is expanded or puffed tobacco. Included herein are methods to produce reduced-exposure tobacco products by utilizing the tobacco provided herein, deproteinized tobacco fiber, and freeze dried tobacco in any combination and in conjunction with expanded or puffed tobacco. More than 150 patents have been issued related to tobacco expansion (*e.g.,* U.S. Patent Number 3,991,772, herein expressly incorporated by reference in its entirety). "Expanded tobacco" is an important part of tobacco filler, which is processed through expansion of suitable gases so that the tobacco is "puffed" resulting in reduced density and greater filling capacity. It reduces the weight of tobacco used in cigarettes. Advantageously, expanded tobacco reduces tar, nicotine and carbon monoxide deliveries and finds use, for example, in making low tar, low nicotine, and low carbon monoxide delivery cigarettes. Expanded tobacco is particularly useful in making low-tar delivery cigarettes. Carlton® cigarettes, which have had claims of being the lowest tar and nicotine delivery cigarette, are reportedly made with a very large percentage of expanded tobacco. Any method for expansion of tobacco known in the art can be used in the methods provided herein. The most common method used today incorporates liquid carbon dioxide (U.S. Patent Nos. 4,340,073 and 4,336,814, herein expressly incorporated by reference in their entireties). Liquid propane has also been used for making commercial cigarettes, predominantly in Europe (U.S. Patent No. 4.531,529, herein expressly incorporated by reference in its entirety). Liquid propane offers advantages over carbon dioxide since higher 3Q degrees of expansion are possible, in the range of 200%. Under

pressure, the liquid carbon dioxide (or liquid propane) permeates the tobacco cell structure. When the tobacco is rapidly heated the carbon dioxide (or liquid propane) expands the cell back to its pre-cured size.

[0090] Another form of modified tobacco is reconstituted tobacco. Included herein are methods to produce reduced-exposure tobacco products by utilizing the tobacco provided herein, deproteinized tobacco fiber, and freeze dried tobacco in any combination and in conjunction with reconstituted tobacco. "Reconstituted tobacco" ("Recon") is an important part of tobacco filler made from tobacco dust and other tobacco scrap material, processed into sheet form and cut into strips to resemble tobacco. In addition to the cost savings, reconstituted tobacco is very important for its contribution to cigarette taste from processing flavor development using reactions between ammonia and sugars.

[0091] The process to produce sheets of Recon began during the 1950s. U.S. Patents that describe such processes include: U.S. Pat. Nos. 3,499,454, 4,182,349 4,962,774, and 6,761,175, herein expressly incorporated by reference in their entireties. Recon is traditionally produced from tobacco stems and/or smaller leaf particles in a process that closely resembles a typical paper making process. The tar and nicotine yields of reconstituted tobacco are lower than those from equivalent quantities of whole tobacco leaf. This process entails processing the various tobacco portions that are to be made into Recon. After the Recon sheets are produced they are cut into a size and shape that resembles cut rag tobacco made from whole leaf tobacco. This cut Recon then is mixed with cut-rag tobacco and is ready for cigarette making. Cigarettes can be manufactured with all Recon, no Recon, or any combination thereof. Processes of removing proteins from tobacco, thereby creating "deproteinized tobacco fiber" are known in the art, as exemplified in U.S. Patent Nos. 4,289,147 and 4,347,324, herein expressly incorporated by reference in its entirety. Tobacco fiber is a major byproduct after removing protein. The fibrous remains from deproteinized tobacco can be included in any percentage as an ingredient of Recon. Cigarettes made from deproteinized tobacco have a different taste than conventional cigarettes. However, appropriate amounts of additives, including flavorings and nicotine, can be added to help alleviate this taste deficiency. Extracting tobacco fiber from modified tobacco effectively eliminates virtually all carcinogenic TSNAs in such tobacco, since nitrosamines require relatively high concentrations of nicotine and other alkaloids to form at

detectable levels. Therefore, it can be advantageous to utilize such a tobacco in reduced-risk cigarettes or other tobacco products to further reduce TSNAs. In some embodiments, a low alkaloid (e.g., LA Burley 21 and/or LA Flue-cured 53) is used in order to manufacture reconstituted tobacco as a means for manufacturing recon with low TSNA levels. PAHs are formed from high temperature pyrolysis of amino acids, sugars, paraffins, terpenes, phytosterols, celluloses and other components of tobacco. Most of these components are greatly reduced in tobacco fiber, effectively reducing formation of PAHs. Catechols and phenols, recognized carcinogenic co-factors in cigarette smoke, would also be reduced since low levels of soluble sugar are present in tobacco fiber. Harmful gas phase compounds such as hydrogen cyanide, nitrogen oxides, and carbon monoxide are also reduced when a cigarette containing only tobacco fiber is smoked compared to a cigarette made with whole-leaf tobacco. Hydrogen cyanide is formed from burning proteins and chlorophyll. Nitrogen oxides are formed from burning soluble protein, chlorophyll, nitrates, and alkaloids. These components are not present in significant amounts in deproteinized tobacco. Tobacco fiber has approximately 85 percent less starches and cellulosic material thus reducing the major pyrolytic precursors of carbon monoxide.

[0092] In another embodiment, TSNAs can be virtually eliminated through processing freshly harvested tobacco using lyophilization. This is accomplished by processing freshly harvested tobacco through freeze-drying units located near tobacco farms.

Tobacco blending

[0093] It may be desirable to blend tobacco having varying levels of toxicants so as to create a reduced risk tobacco product with varying taste and/or toxicant characteristics. This blending process is typically performed after the curing process, and may be performed by conventional methods. Preferred tobacco blending approaches are provided below. A mixture that contains different types of tobacco is desirably substantially homogeneous throughout in order to avoid undesirable fluctuations in taste or toxicant levels. Typically, tobacco to be blended may have a moisture content from about 10 to about 40%. As an example, the tobacco is first cut or shredded to a suitable size, then mixed in a mixing device, such as a rotating drum or a blending box. One such known mixing device is a tumbling apparatus that typically comprises a rotating housing enclosing mixing paddles which are

attached to and, therefore, rotate with the housing to stir the tobacco components together in a tumbling action as the drum turns.

[0094] After the desired tobaccos are thoroughly mixed, the resulting tobacco blend is removed from the mixing apparatus and bulked to provide a continuous, generally uniform quantity of the tobacco blend. The tobacco is then allowed to remain relatively undisturbed (termed the "bulking step") for the required period of time before subsequent operations are performed.

[0095] The tobacco blend can be expanded by the application of steam. After the tobacco blend has been expanded, it is dried. The dried, expanded tobacco blend is then in a suitable mode to be processed into a reduced risk product, as described below.

[0096] Some blending approaches begin with tobacco prepared from one or more tobaccos that have a reduced level of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to level of compounds that cause the induction of biological insult induced by a conventional or reference cigarette (e.g., 2R4F)in human cells contacted by smoke from the tobacco. In some blending approaches, one or more tobaccos that have extremely low amounts of nicotine, nornicotine, sterols and/or TSNAs are used (e.g., low alkaloid tobaccos, such as LA Burley 21 and/or LA Flue 53). In some embodiments, the reduced-risk tobacco is blended with one or more conventional tobaccos.

[0097] Typical tobacco blends include a blend of Oriental tobacco, Burley tobacco and Flue cured tobacco. The tobacco products provided herein can contain 0-50%, 5-40%, or 5-25% Oriental tobacco. The tobacco products provided herein can contain 0-90%, 5-80%, 10-80%, 10-70%, 10-60%, or 10-50% Burley tobacco (preferable LA Burley 21). The tobacco products provided herein can contain 0-70%, 5-60%, 5-50%, 10-50%, 10-40%, or 10-30% Flue-cured tobacco (e.g., conventional Flue-cured tobacco or LA Flue 53). An exemplary tobacco product, e.g., a cigarette, contains a blend of 5-25% Oriental tobacco, 10-50% Burley and 10-50% Flue cured. In particular embodiments provided herein, a blend of Oriental tobacco, Burley tobacco and Flue cured tobacco is provided, where at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90%, or all, of the Burley tobacco is a Burley tobacco cured to have a reduced level of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or

proteome as compared to the level of compounds that cause biological insult induced by a conventional or reference cigarette (e.g., 2R4F), in human cells contacted by smoke from the tobacco (e.g., LA Burley 21).

[0098] In one embodiment, conventional Flue-cured bright tobacco is blended with a low alkaloid Burley (e.g., LA Burley 21), and without conventional Burley, to yield a blended reduced-risk tobacco. In another embodiment, conventional Flue-cured tobacco and Oriental tobacco is blended with a low alkaloid Burley (e.g., LA Burley 21). Optionally, conventional Burley and/or a low alkaloid Flue tobacco (e.g., LA Flue 53) can be used to yield a blended reduced-risk tobacco.

[0099] In an embodiment, the tobacco blend comprises Flue-cured tobacco, Burley tobacco, and Oriental tobacco. In an embodiment, the Flue-cured tobacco is present in an amount from about 1% to about 15% by weight of the total blend. In an embodiment, the Flue-cured tobacco is present in an amount from about 5% to about 10% by weight of the total blend, including, for example, about 6-7% by weight of the total blend. In an embodiment, the Burley tobacco is present in an amount from about 50% to about 70% by weight of the total blend. In an embodiment, the Burley tobacco is present in an amount from about 55% to about 65% by weight of the total blend, including, for example about 58-60% by weight of the total blend. In an embodiment, a portion of the Burley tobacco comprises low-alkaloid Burley tobacco (e.g., LA Burley 21). In an embodiment, low alkaloid Burley tobacco is present in an amount from about 5% to about 25% by weight of the total blend. In an embodiment, low alkaloid Burley tobacco is present in an amount from about 50% to about 70% by weight of the total blend, including, for example, about 55-65% by weight of the total blend. In an embodiment, the Oriental tobacco is present in an amount from about 5% to about 25% by weight of the total blend. In an embodiment, the Oriental tobacco is present in an amount from about 10% to about 20% by weight of the total blend, including, for example about 11-15% by weight of the total blend. In an embodiment, the tobacco blend further comprises cut rolled expanded stem. In an embodiment, the cut rolled expanded stem is present in an amount from about 5% to about 25% by weight of the total blend. In an embodiment, the cut rolled expanded stem is present in an amount from about 15% to about 25% by weight of the total blend, including, for example, about 22% by weight of the total blend. In an exemplary embodiment, the tobacco blend comprises about 7%

Flue-cured tobacco, about 59% Burley tobacco, about 12% Oriental tobacco, and about 22% cut rolled expanded stem. In an embodiment, a portion of the Burley tobacco is low alkaloid Burley tobacco (e.g., LA Burley 21), which is present in an amount of about 59% by weight of the total blend.

[0100] It should be appreciated that tobacco products are often a blend of many different types of tobaccos, which were grown in many different parts of the world under various growing conditions. As a result, the amount of nicotine, TSNAs, sterols, PAHs and other toxicant may differ from crop to crop. Nevertheless, by using conventional techniques one can easily determine an average amount of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells, and/or an average amount of nicotine, TSNA, sterol, PAH or any toxicant per crop used to create a desired blend. It should also be appreciated that reconstituted, expanded, chemically treated, or microbial treated tobacco can be blended with the modified tobacco described herein. By adjusting the amount of each type of tobacco that makes up the blend one of skill can balance the amount of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells, nicotine, TSNA, sterol and/or PAH with other considerations such as appearance, flavor, and smokability. In this manner, a variety of types of tobacco products having varying level of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells, nicotine, TSNA, PAH, or other toxicants as well as, appearance, flavor and smokability can be created.

Filters

[0101] In some embodiments, the cigarette filters used with the cigarettes described herein comprise a carbon and/or an ion-exchange resin.

Activated Carbon in Filters

[0102] Activated carbon in a cigarette filter for selective removal of vapor phase smoke constituents has been utilized for over almost four decades. Commercial products have utilized a wide range of activated carbon types with various properties and significant effort has been used to identify materials that provide specificity for removal of toxic constituents while maintaining acceptable sensory properties during smoking. Selective

filtration occurs through a vapor phase molecule interacting with an active site on the carbon though a physical adsorption or chemical adsorption mechanism. During the smoking process this molecule is in equilibrium with the smoke particle and the gas phase. In addition, the vapor phase molecule is in equilibrium with the surfaces inside the cigarette filter. The cellulose acetate filter material has a weak binding affinity for vapor phase molecules therefore the equilibrium is shifted toward the gas phase. Carbon, silica gel, zeolites, resins and other common filter additives, however, have a stronger affinity for vapor phase molecules and the equilibrium is shifted toward the molecule residing on the surface of the filter additive. The vapor phase molecule structure and the surface of the filter additive have a significant effect on filtration. This structure and surface affect whether the material is retained through a physical adsorption process or a chemical process.

[0103] More recent developments have identified that carbons with higher carbon tetrachloride activity provide increased removal efficiency for vapor phase constituents including 1,3-butadiene, isoprene, acetaldehyde, acetone, acrylonitrile, benzene and butyraldehyde etc. (US 2007/0261706A1 to Banerjea et. al.). Numerous studies have indicated that carbon loses its efficiency to remove specific vapor phase components from smoke as the product ages. This has been attributed to depletion of active sites by triacetin in the filter. In order to compensate, cigarette designs have incorporated higher activity carbons and higher levels of activated carbon in the filter to achieve the same removal efficiency. Activated beaded carbons have also been proposed (US 2003/0154993A1 to Paine et al.). Beaded activated carbons have been selected based on their activities and surface area determined using the BET (Brunauer, Emmet and Teller) method. These beaded carbons have been manufactured with a well defined micropore and mesopore distribution of 50 angstrom in diameter and identified as optimal performance measures.

[0104] The surface structure of carbon has been very well characterized and consists of an amorphous structure containing a series of mesopores, micropores and super micropores. These pores are characterized based on their pore volume and pore diameter. Surface area as measured by the BET method is a measure of the amorphous portions of the surface plus the interior volumes collectively of the series of pore types. Pore volume can be measured but is generally not utilized with conventional applications and is not provided by activated carbon manufacturers. Depending upon the manner in which the carbon is

activated, the pore volume can vary dramatically. Carbons with the same surface area can have vastly differing pore volumes. Sasaki et al. examined the effect of pore size and pore volume on adsorption of acetone and other volatiles in cigarette smoke. (Sasaki, T.; Matsumoto, A.; Yamashita, Y. "The effects of pore size and volume of activated carbon on adsorption efficiency of vapor phase compounds in cigarette smoke" Colloids and Surfaces A 325 (2008) 166-172.) In general, adsorption tracked surface area. We have discovered a unique feature not revealed by the prior art, namely, correlation between pore volume and biological insult. Pore volume has significantly more effect on reducing the induction of biological insult on removal than surface area.

[0105] In one embodiment, of the current invention, a carbon is selected based on pore volume. Higher pore volume carbons having the same surface area and lower pore volume carbons will allow increased removal efficiency of vapor phase smoke constituents. The lowering of vapor phase components in smoke leads to less DNA damage resulting in reduced biological effects. The usage of this approach allows cigarette designs to provide higher reductions in unwanted smoke components by using lower quantities of carbon in the cigarette filter. The use of lower quantities provides a cigarette with increased sensory perception and more consumer acceptability.

[0106] Theoretically, the behavior of the carbon is based on the equilibrium reaction:

Smoke Particle $\leftrightarrow$ Vapor Phase $\leftrightarrow$ Carbon Surface

[0107] In order to remove the material from smoke and protect the smoker from exposure to volatile constituents the equilibrium must be driven toward the right (carbon surface). Selection of a carbon that retains the vapor phase components and does not allow them to re-enter the vapor phase provide reduced deliveries of these constituents in smoke and therefore less biological damage. A portion of the molecules that are adsorbed onto the outer surface of the carbon are readily desorbed back into the smoke flow and delivered to the smoker, shifting the equilibrium to the left. Pores behave differently. As molecules enter the pore and adsorb to the walls inside the pore, the probability of desorption and re-entry into the main flow of smoke is diminished as it is more likely the molecule contact another location within the pore. As the pore becomes larger this probability is more likely. During the smoking process, the pores begin to fill leading to lower level of vapor phase removal in

later puffs. Increase in pore volume allows better removal efficiency for the later puffs. Pore diameter (as specified in US 2003/0154993A1 to Paine et al.) becomes more important for smaller diameter pores due to the potential for adsorbed molecules to block the opening to the pore thereby limiting removal in later puffs. In regards to larger diameter pores, >50 nm as specified in (US 2003/0154993A1 to Paine et al.) molecules that adsorb closer to the pore entrance have an increased probability to desorb and return to the mainstream smoke flow.

[0108] In one embodiment, the pore volume of a carbon is 0.1 mL/g – 0.9 mL/g, such as 0.2 mL/g – 0.8 mL/g, 0.3 mL/g – 0.7 mL/g, 0.4 mL/g – 0.6 mL/g, or about 0.5 mL/g, based on a nitrogen adsorptive anaylsis. In other embodiments, the pore volume of a carbon is 0.1 mL/g – 0.8 mL/g, 0.1 mL/g – 0.7 mL/g, 0.1 mL/g – 0.6 mL/g, 0.1 mL/g – 0.5 mL/g, 0.1 mL/g – 0.4 mL/g, 0.1 mL/g – 0.3 mL/g, 0.1 mL/g – 0.2 mL/g, 0.2 mL/g – 0.9 mL/g, 0.2 mL/g – 0.8 mL/g, 0.2 mL/g – 0.7 mL/g, 0.2 mL/g – 0.6 mL/g, 0.2 mL/g – 0.5 mL/g, 0.2 mL/g – 0.4 mL/g, 0.2 mL/g – 0.3 mL/g, 0.3 mL/g – 0.9 mL/g, 0.3 mL/g – 0.8 mL/g, 0.1 mL/g – 0. 7 mL/g, 0.3 mL/g – 0.6 mL/g, mL/g, 0.3 mL/g – 0.5 mL/g, mL/g, 0.3 mL/g – 0.4 mL/g,– 0.9 mL/g, 0.4 mL/g,– 0.8 mL/g, 0.4 mL/g,– 0.7 mL/g, 0.4 mL/g,– 0.6 mL/g, 0.4 mL/g,– 0.5 mL/g, 0.5 mL/g,– 0.9 mL/g, 0.5 mL/g,– 0.8 mL/g, 0.5 mL/g,– 0.7 mL/g, 0.5 mL/g,– 0.6 mL/g, 0.6 mL/g,– 0.9 mL/g, 0.6 mL/g,– 0.8 mL/g, 0.6 mL/g,– 0.7 mL/g, 0.7 mL/g,– 0.9 mL/g, 0.7 mL/g,– 0.8 mL/g, or 0.8 mL/g,– 0.9 mL/g.

[0109] In another embodiment, the total pore volume distribution of a carbon is from 0.1 mL/g – 0.9 ml/g such as 0.2 mL/g – 0.8 mL/g, 0.3 ml/g – 0.7 mL/g, 0.4 mL/g – 0.6 mL/g, wherein the percentage of a carbon having the total pore volume distribution is at least 50% in one embodiment, and in other embodiments the percentage of carbon having the total pore volume distribution is 50-90%, 50-85%, 50-80%, 50-75%, 50-70%, 50-65%, 50-60%, 50-55%, 55-90%, 55-85%, 55-80%, 55-75%, 55-70%, 55-65%, 55-60%, 60-90%, 60-85%, 60-80%, 60-75%, 60-70%, 60-65%, 65-90%, 65-85%, 65-80%, 65-75%, 65-70%, 70-90%, 70-85%, 70-80%, 70-75%, 75-90%, 35-85%, 75-80%, 80-90%, 80-85%, or 85-90%. In other embodiments, the pore volume distribution of a carbon is 0.1 mL/g – 0.8 mL/g, 0.1 mL/g – 0.7 mL/g, 0.1 mL/g – 0.6 mL/g, 0.1 mL/g – 0.5 mL/g, 0.1 mL/g – 0.4 mL/g, 0.1 mL/g – 0.3 mL/g, 0.1 mL/g – 0.2 mL/g, 0.2 mL/g – 0.9 mL/g, 0.2 mL/g – 0.8 mL/g, 0.2 mL/g – 0.7 mL/g, 0.2 mL/g – 0.6 mL/g, 0.2 mL/g – 0.5 mL/g, 0.2 mL/g – 0.4 mL/g, 0.2 mL/g – 0.3 mL/g, 0.3 mL/g – 0.9 mL/g, 0.3 mL/g – 0.8 mL/g, 0.3 mL/g – 0.7 mL/g, 0.3 mL/g – 0.6

mL/g, mL/g, 0.3 mL/g – 0.5 mL/g, mL/g, 0.3 mL/g – 0.4 mL/g, 0.4 mL/g – 0.9 mL/g, 0.4 mL/g,– 0.8 mL/g, 0.4 mL/g,– 0.7 mL/g, 0.4 mL/g,– 0.6 mL/g, 0.4 mL/g,– 0.5 mL/g, 0.5 mL/g,– 0.9 mL/g, 0.5 mL/g,– 0.8 mL/g, 0.5 mL/g,– 0.7 mL/g, 0.5 mL/g,– 0.6 mL/g, 0.6 mL/g,– 0.9 mL/g, 0.6 mL/g,– 0.8 mL/g, 0.6 mL/g,– 0.7 mL/g, 0.7 mL/g,– 0.9 mL/g, 0.7 mL/g – 0.8 mL/g, or 0.8 mL/g,– 0.9 mL/g, wherein the percentage of a carbon having each of the total pore volume distributions above is at least 50% in one embodiment, and in other embodiments, the percentage of carbon having each of the total pore volume distributions above is 50-90%, 50-85%, 50-80%, 50-75%, 50-70%, 50-65%, 50-60%, 50-55%, 55-90%, 55-85%, 55-80%, 55-75%, 55-70%, 55-65%, 55-60%, 60-90%, 60-85%, 60-80%, 60-75%, 60-70%, 60-65%, 65-90%, 65-85%, 65-80%, 65-75%, 65-70%, 70-90%, 70-85%, 70-80%, 70-75%, 75-90%, 35-85%, 75-80%, 80-90%, 80-85%, or 85-90%.

[0110] In another embodiment, the pore size measured as the diameter of the pore is, such as 0.6 nm – 1.0 nm, 0.7 nm – 0.9 nm or 0.8 nm to 0.9 nm. In another embodiment, a filter comprising carbon having a combination of the pore size and pore volume is used. For example, 0.1 mL/g – 0.9 mL/g pore volume and 0.5 nm – 1.1 nm pore diameter.

[0111] Activated carbon, such as TA95, which may be used in the filters described herein may have adsorption capacity for acetone of 0.3 mL/g – 0.9 mL/g, such as 0.4 mL/g – 0.8 mL/g, 0.5 mL/g – 0.7 mL/g.

[0112] Other materials such as silica gel, zeolites, resins etc. have slightly different surface structures and less pore structure. Removal of vapor phase constituents and retention on these materials relies more heavily on chemical adsorption such as hydrogen bonding effects or chemical reaction whereas carbon relies more heavily on London forces and with aromatic molecules, "pπ-pπ" interactions of graphite like portions of the carbon surface.

Forms of Filters

[0113] Filters comprising a carbon, ion-exchange resins, or a combination thereof can take several forms and the manner in which the carbon and/or ion-exchange resin is incorporated into the cigarette filter may vary. In one embodiment, the filter is a tripartite design. In one tripartite design, the court in its downstream from the resident in the filter, for example the cigarette will have a cellulose acetate filter portion, a portion containing carbon, and a portion containing resin, immediately before the tobacco. Another tripartite design

comprises three compartments for containing carbon, weak base amine-containing resin, and sepiolite, or any acceptable adsorbent material capable of removing volatile and/or semi-volatile constituents from the vapor phase of cigarette mainstream smoke.

[0114] In one embodiment, it was found that the a mixture of sepiolite and an ion exchange resin yielded a filter that was significantly more effective in reducing toxicants causing biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome, than a filter containing the same amount of the either or sepiolite or resin by weight. Without being bound by a theory, it is believed that the combination with sepiolite allows the resin to be more uniformly dispersed throughout the filter segment as well as assists sepiolite's ability to adsorb and subsequently desorb volatile components from the mainstream cigarette smoke ultimately providing longer residence time for the volatile components to desorb from the sepiolite and irreversibly bind to the weak-base primary amine resin.

[0115] In an embodiment, the cigarette filter comprises a Dalmatian-type cigarette filter. For example, the filter can comprise a Dalmatian-type filter, wherein the carbon and/or ion-exchange resin is distributed throughout the filter in a uniform manner. In some embodiments, the filter comprises carbon in a particulate form, wherein the carbon particles are dispersed throughout the filter uniformly. In some embodiments, the filter comprises an ion-exchange resin in particulate form, wherein the ion-exchange resin particles are dispersed throughout the filter uniformly. In more embodiments, the filter comprises carbon in a particulate form and ion-exchange resin in a particulate form and both the carbon particles and the ion-exchange resin particles are dispersed throughout the filter uniformly.

[0116] The carbon and/or ion-exchange resin can be dispersed within a material that makes up the filter. The filter can be made from a variety of materials. For example, the filter may comprise any filter material known for use in cigarette filters. In some embodiments, the cigarette filter is manufactured from cellulose acetate tow, gathered cellulose acetate web, polypropylene tow, gathered polypropylene web, gathered polyester web, gathered paper, or combinations thereof.

[0117] In some embodiments, the cigarette filter comprises multiple sections. A filter section can comprise any portion of the cigarette filter, for example, the filter section may comprise a longitudinally extending section within the filter. In some embodiments, the

filter comprises at least a first longitudinally extending section of filter material positioned at the end of the filter proximal to the tobacco rod (i.e., tobacco end section) and at least a second longitudinally extending section of filter material positioned at the end of the filter element distal to the tobacco rod (i.e., mouth end section). The number and type of sections in the filter can vary over a wide range, including one, two, three, four, five, six, or seven sections, wherein any one section can be the same or different from any other section.

[0118] One or more sections of a multiple section filter can comprise a Dalmatian-type section, wherein the carbon and/or ion-exchange resin is uniformly dispersed within that section. Additionally, one or more sections of a multiple section filter can comprise carbon and/or ion-exchange resin in concentrated amounts, rather than being uniformly dispersed throughout the filter.

[0119] In some embodiments, the cigarette filter comprises one longitudinally extending section that is a Dalmatian-type section, wherein the carbon and/or ion-exchange resin is uniformly dispersed within that section. In some embodiments, the cigarette filter comprises two longitudinally extending sections that are a Dalmatian-type section, wherein the carbon and/or ion-exchange resin is uniformly dispersed within those sections. In some embodiments, the cigarette filter comprises three longitudinally extending sections that are a Dalmatian-type section, wherein the carbon and/or ion-exchange resin is uniformly dispersed within those sections. In some embodiments, the cigarette filter comprises four longitudinally extending sections that are a Dalmatian-type section, wherein the carbon and/or ion-exchange resin is uniformly dispersed within those sections.

[0120] In some embodiments, the cigarette filter comprises one longitudinally extending section comprising carbon and/or ion-exchange resin in a concentrated amount. In some embodiments, the cigarette filter comprises two longitudinally extending sections that each comprise carbon and/or ion-exchange resin in a concentrated amount. In some embodiments, the cigarette filter comprises three longitudinally extending sections that each comprise carbon and/or ion-exchange resin in a concentrated amount. In some embodiments, the cigarette filter comprises four longitudinally extending sections that each comprise carbon and/or ion-exchange resin in a concentrated amount.

[0121] Figure 1 shows an illustrative embodiment of a cigarette filter 230 comprising three sections 132, 134, 136. The mouth-end section 132 can comprise any

material suitable for manufacturing cigarette filters, for example, cellulose acetate. In some embodiments, the mouth-end section comprises cellulose, cellulose acetate tow, paper, cotton, polypropylene web, polypropylene tow, polyester web, polyester tow or combinations thereof. In some embodiments, the mouth-end section includes a plasticizer. In some embodiments, the mouth-end section comprises carbon, ion-exchange resin, or a combination thereof. In more embodiments, carbon, ion-exchange resin, or a combination thereof is absent from the mouth-end section.

[0122] Cigarette filter sections 134 and 136 can be the same or different as the mouth-end section 132, and some embodiments comprise cellulose, cellulose acetate tow, paper, cotton, polypropylene web, polypropylene tow, polyester web, polyester tow or combinations thereof. Furthermore, sections 134 and 136 can be the same or different. In some embodiments, the filter section 134 adjacent the tobacco rod comprises an adsorbent 144. In some embodiments, the adsorbent 144 is carbon, ion-exchange resin, or a combination thereof. In some embodiments, the middle filter section 136 comprises an adsorbent 146. In some embodiments, the adsorbent 146 is carbon, ion-exchange resin, or a combination thereof.

[0123] In some embodiments, the cigarette filter comprises two sections. In some embodiments, the mouth-end section of a two-section filter comprises cellulose, cellulose acetate tow, paper, cotton, polypropylene web, polypropylene tow, polyester web, polyester tow or combinations thereof. In some embodiments, the mouth-end section of a two-section filter comprises cellulose acetate. In some embodiments, the tobacco-end section of a two-section filter comprises cellulose, cellulose acetate tow, paper, cotton, polypropylene web, polypropylene tow, polyester web, polyester tow or combinations thereof. In some embodiments, the tobacco-end section of a two-section filter comprises cellulose acetate. In some embodiments, the tobacco-end section of a two-section filter comprises carbon, ion-exchange resin, or a combination thereof. In some embodiments, the tobacco-end section of a two-section filter is a Dalmatian-type section and comprises carbon, ion-exchange resin, or a combination thereof.

[0124] The length of the cigarette filter can vary over a wide range. In some embodiments, the cigarette filter has a length of about 15 mm to about 65 mm. In some embodiments, the cigarette filter has a length of about 20 mm to about 40 mm. In some

embodiments, the cigarette filter has a length of about 20 mm to about 30 mm. In some embodiments, the cigarette filter has a length of about 25 mm to about 35 mm. In some embodiments, the cigarette filter has a length of about 25 mm. In some embodiment, the cigarette filter has a length of about 30 mm.

[0125] Where the filter comprises multiple sections, the length of each section can be selected to provide optimal performance for reducing biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells. The total length of the combined segments results in a cigarette filter having a length of about 15 mm to about 65 mm, as discussed above. In some embodiments, a filter section in a multiple section filter has a length of about 1 mm to about 65 mm. In some embodiments, a filter section in a multiple section filter has a length of about 1 mm to about 40 mm. In some embodiments, a filter section in a multiple section filter has a length of about 1 mm to about 30 mm. In some embodiments, a filter section in a multiple section filter has a length of about 1 mm to about 25 mm. In some embodiments, a filter section in a multiple section filter has a length of about 1 mm to about 20 mm. In some embodiments, a filter section in a multiple section filter has a length of about 1 mm to about 15 mm. In some embodiments, a filter section in a multiple section filter has a length of about 1 mm to about 10 mm. In some embodiments, a filter section in a multiple section filter has a length of about 1 mm to about 7 mm. In some embodiments, a filter section in a multiple section filter has a length of about 1 mm to about 5 mm. In some embodiments, a filter section in a multiple section filter has a length of about 4 mm to about 10 mm. In an embodiment, a filter section in a multiple section filter has a length of about 7 mm to about 15 mm. In some embodiments, a filter section in a multiple section filter has a length of about 12 mm to about 20 mm.

[0126] In some embodiments comprising two sections, the mouth-end section of the filter has a length of about 1 mm to about 12 mm and the tobacco-end section of the filter has a length of about 10 mm to about 40 mm. In some embodiments comprising two sections, the mouth-end section of the filter has a length of about 4 mm to about 10 mm and the tobacco-end section of the filter has a length of about 12 mm to about 30 mm. In some embodiments comprising two sections, the mouth-end section of the filter has a length of about 6 mm to about 8 mm and the tobacco-end section of the filter has a length of about 15 mm to about 20 mm. In some embodiments comprising two sections, the mouth-end section

of the filter has a length of about 7 mm and the tobacco-end section of the filter has a length of about 18 mm. In some embodiments comprising three sections, the mouth-end section of the filter has a length of about 1 mm to about 12 mm, the middle filter section has a length of about 1 mm to about 20 mm, and the tobacco-end section of the filter has a length of about 1 mm to about 12 mm. In some embodiments comprising three sections, the mouth-end section of the filter has a length of about 4 mm to about 10 mm, the middle filter section has a length of about 4 mm to about 16 mm, and the tobacco-end section of the filter has a length of about 4 mm to about 10 mm. In some embodiments comprising three sections, the mouth-end section of the filter has a length of about 8 mm to about 12 mm, the middle filter section has a length of about 10 m to about 14 mm, and the tobacco-end section of the filter has a length of about 8 mm to about 12 mm. In some embodiments comprising three sections, the mouth-end section of the filter has a length of about 9 mm, the middle filter section has a length of about 12 mm, and the tobacco-end section of the filter has a length of about 9 mm.

[0127] Any of a variety of multiple section cigarette filters known in the art can be used in the compositions and methods provided herein, for example, those multiple section cigarette filters discussed in U.S. Patent No. 6,779,529 to Figlar et al. and U.S. Patent Application Publication No. 2004/0237984 to Figlar, the contents of which are hereby incorporated by reference in their entirety. In some embodiments, a cigarette having a multiple section filter comprises a section containing a general adsorbent, such as carbon, and a section containing a selective adsorbent, such as ion-exchange resin is used.

[0128] The first and second longitudinally extending sections can be the same or different. Additional longitudinally extending filter sections are also contemplated. In some embodiments, the filter comprises three or more longitudinally extending filter sections. In some embodiments, the filter comprises four or more longitudinally extending filter sections. In some embodiments, the filter comprises five or more longitudinally extending filter sections. Each filter section may be the same or different from any other filter section. For example, a first longitudinally extending section can be different from a second longitudinally extending section, but similar to a third longitudinally extending section. Various combinations of filter sections are contemplated herein. In some embodiments, an adsorbent material, such as carbon or ion-exchange resin, is contained within at least a portion of at least one of the longitudinally extending sections. In some embodiments, the

carbon is present in one, two, three, four, or five sections of a multiple section filter. In some embodiments, the ion-exchange resin is present in one, two, three, four, or five sections of a multiple section filter.

[0129] Any of a variety of cigarette filters comprising cavities and/or channels known in the art can be used in the compositions and methods provided herein, for example, those cigarette filters comprising cavities and/or channels discussed in U.S. Patent Nos. 7,240,678 to Crooks et al. and 7,237,558 to Clark et al., the contents of which are hereby incorporated by reference in their entirety.

[0130] Any section of a multiple section filter may comprise a cavity. Filter cavities may be filled with carbon and/or ion-exchange resin and allow larger amounts of the carbon and/or ion-exchange resin to be present in the section that comprises the cavity. In some embodiments, a multiple section filter comprises one, two, three, four, or five sections that comprise a cavity. In some embodiments, a filter section that comprises a cavity further comprises carbon, ion-exchange resin, or a combination thereof. Multiple section filters comprising at least one cavity are well known in the art, and are sometimes referred to as a "compartment filter" or a "plug/space/plug" filter.

[0131] A filter section comprising a cavity can have a variety of lengths. In some embodiments, the length of the cavity is from 1 mm to about 20 mm. In some embodiments, the length of the cavity is from about 3 mm to about 12 mm. In some embodiments, the length of the cavity is from about 5 mm to about 10 mm. In some embodiments, the length of the cavity is about 5 mm. In an embodiment, the length of the cavity is about 7 mm. In an embodiment, the length of the cavity is about 9 mm. In some embodiments, the length of the cavity is about 10 mm. In some embodiments, the length of the cavity is about 12 mm.

[0132] The carbon and/or ion-exchange resin can be provided in varying sectional amounts within the various sections of the filter. For example any longitudinal section can comprise more or less of an amount of carbon and/or ion-exchange resin than any other filter section of the multiple-section filter. In some embodiments, the filter element comprises a first section of filter material, such as a fibrous filter material (e.g., plasticize cellulose acetate tow) and a second section of filter material spaced apart from the first section of filter material. The first section of filter material is positioned at the mouth end of the filter element and the second section of filter material is positioned proximal to the tobacco rod.

The space between the first section of filter material and the second section of filter material is a third section and defines a cavity. At least a portion of the cavity contains an adsorbent material, such as carbon, ion-exchange resin, or a combination thereof, either in beaded granular form. Typically, substantially the entire compartment contains an adsorbent, such as carbon, ion-exchange resin, or a combination thereof.

[0133] In some embodiments, the cavity comprises carbon and/or ion-exchange resin an amount from about 20 mg to about 300 mg. In some embodiments, the cavity comprises carbon and/or ion-exchange resin an amount from about 40 mg to about 250 mg. In some embodiments, the cavity comprises carbon and/or ion-exchange resin an amount from about 60 mg to about 200 mg. In some embodiments, the cavity comprises carbon and/or ion-exchange resin an amount from about 80 mg to about 180 mg. In some embodiments, the cavity comprises carbon and/or ion-exchange resin an amount from about 90 mg to about 160 mg. In some embodiments, the cavity comprises carbon and/or ion-exchange resin an amount from about 95 mg to about 140 mg. In some embodiments, the cavity comprises carbon and/or ion-exchange resin an amount from about 110 mg to about 130 mg. In some embodiments, the cavity comprises carbon and/or ion-exchange resin an amount of about 95 mg. In some embodiments, the cavity comprises carbon and/or ion-exchange resin an amount of about 124 mg. In some embodiments, the cavity comprises carbon and/or ion-exchange resin an amount of about 137 mg. In some embodiments, the cavity comprises carbon and/or ion-exchange resin an amount of about 144 mg. In some embodiments, the cavity comprises carbon and/or ion-exchange resin an amount of about 179 mg.

[0134] In a multiple section filter comprising a cavity, wherein the cavity comprises carbon and/or ion-exchange resin, other filter sections besides the cavity section my also comprise an amount of carbon and/or ion-exchange resin. In some embodiments, the non-cavity section comprises carbon and/or ion-exchange resin in an amount from about 20 mg to about 200 mg. In some embodiments, the non-cavity section comprises carbon and/or ion-exchange resin in an amount from about 40 mg to about 100 mg. In some embodiments, the non-cavity section comprises carbon and/or ion-exchange resin in an amount from about 50 mg to about 80 mg. In some embodiments, the non-cavity section comprises carbon and/or ion-exchange resin in an amount of less than or equal to 40 mg. In some

embodiments, the non-cavity section comprises carbon and/or ion-exchange resin in an amount of less than or equal to 50 mg. In some embodiments, the non-cavity section comprises carbon and/or ion-exchange resin in an amount of less than or equal to 68 mg.

[0135] In some embodiments, the cigarette filter comprises a mouth-end section, a cavity, and a tobacco-end section. In some embodiments, the mouth-end section comprises cellulose acetate. In some embodiments, the cavity section comprises carbon, ion-exchange resin, or a combination thereof. In some embodiments, the tobacco-end section is a Dalmatian-type section that comprises carbon, ion-exchange resin, or a combination thereof.

[0136] In some embodiments, the cigarette filter comprises channels within the filter. Filter channels can extend radially or longitudinally within the cigarette filter. Channels can also be present in filters that comprise a single section and filters that comprise multiple sections. In multiple section filters, a channel can extend throughout a single section of the filter, throughout multiple sections (*e.g.* two, three, four, five, etc.) of the filter, or throughout all of the sections of the filter.

[0137] In some embodiments, at least one channel extends through the tobacco end section of filter material, the channel being adapted for passage of mainstream smoke between the tobacco rod and the compartment containing the adsorbent material. In some embodiments, a single channel extends through the tobacco end section of filter material or a plurality of channels can be utilized. In one embodiment, a single channel proximal to the central axis of the tobacco end section of filter material is used. In other embodiments, a plurality of channels extend through the filter material, either spaced along the periphery of the filter material or grouped in the area proximal to the central axis of the tobacco end section of filter material. The total cross-sectional area of the one or more channels extending through the first section of filter material may be about 0.1 to about 50 mm^2, preferably about 0.5 to about 15 mm^2.

[0138] The cross-sectional shape of the channels is not critical to the invention and may be, for example, rectangular or circular. The diameter of each channel or tube can vary. Typically, the diameter of each channel or tube is about 0.5 to about 8 mm, frequently about 1 to about 3 mm. The diameter of the channel or tube is selected so as to prevent migration of the adsorbent into the channel or tube (i.e., the diameter of the channel or tube is smaller than the diameter of the adsorbent particles).

[0139] In some embodiments, the filter element is attached to the tobacco rod by a tipping material, which circumscribes both the entire length of the filter element and an adjacent region of the tobacco rod. The inner surface of the tipping material can be fixedly secured to the outer surface of the plug wrap and the outer surface of the wrapping material of the tobacco rod using a suitable adhesive. In some embodiments, a ventilated or air diluted smoking article is provided a series of perforations for air dilution. In some embodiments, each of the perforations extend through the tipping material and the plug wrap. In some embodiments, the filter element is ventilated to provide a cigarette having an air dilution from about 0 to about 75 percent. In some embodiments, the filter element is ventilated to provide a cigarette having an air dilution from about 15 to about 65 percent. In some embodiments, the filter element is ventilated to provide a cigarette having an air dilution from about 25 to about 40 percent. "Air dilution" is the ratio (expressed as a percentage) of the volume of air drawn through the perforations to the total volume of air and smoke drawn through the cigarette and exiting the extreme mouth end portion of the cigarette. The perforations can be made by various techniques known to those of ordinary skill in the art. For example, the perforations can be made using mechanical or microlaser offline techniques or using online laser perforation. In some embodiments, the tipping paper circumscribes the entire filter element and about 4 mm of the length of the tobacco rod in the region adjacent to the filter element.

[0140] Cigarette filter elements that incorporate adsorbents, such as carbon, ion-exchange resin, or combinations thereof, have a propensity to remove certain gas phase components from the mainstream smoke that passes through the filter element during draw by the smoker. The interaction of mainstream smoke with adsorbent substances results in a certain degree of removal of certain gas phase compounds from the smoke. Adsorbents within a cigarette filter are capable of removing a multitude of compounds, including carbonyl compound, including, but not limited to, acetone, formaldehyde, acrolein and acetaldehyde. In some embodiments, the adsorbents are carbon, ion-exchange resin, or a combination thereof. Examples of adsorbents useful herein include activated charcoal, activated coconut carbon, activated coal-based carbon, zeolite, silica gel, meerschaum, aluminum oxide, or combinations thereof.

[0141] In embodiments in which the filter comprises carbon, the amount and type of carbon in the filter can vary over a wide range. The amount of carbon within the filter typically ranges from about 5 to about 240 mg, and a person having ordinary skill in the art would understand that any range within this broader range is contemplated by the inventors. In some embodiments, the carbon within the filter ranges from about 20 to about 120 mg. In some embodiments, the carbon within the filter ranges from about 40 to about 108 mg. In some embodiments, the carbon within the filter comprises about 40, about 60, about 80, or about 108 mg.

[0142] Narrower ranges of smaller amounts of carbon are also contemplated. In some embodiments, the carbon within the filter ranges from about 35 to about 45 mg. In some embodiments, the carbon within the filter ranges from about 55 to about 65 mg. In some embodiments, the carbon within the filter ranges from about 75 to about 85 mg. In some embodiments, the carbon within the filter ranges from about 100 to about 115 mg. In some embodiments, the carbon within the filter ranges from about 110 to about 130 mg. In some embodiments, the carbon within the filter ranges from about 30 to about 70 mg. In some embodiments, the carbon within the filter ranges from about 20 to about 50 mg. For example, the carbon within the filter can be about 20 mg.

[0143] Narrower ranges of larger amounts of carbon are also contemplated. In some embodiments, the carbon within the filter ranges from about 150 to about 250 mg. In some embodiments, the carbon within the filter ranges from about 100 to about 200 mg. In some embodiments, the carbon within the filter ranges from about 200 to about 250 mg. In some embodiments, the carbon within the filter ranges from about 100 to about 150 mg. For example, the carbon within the filter can be about 120 mg.

[0144] The carbon incorporated into the filter of the cigarette can vary among a number of types and sizes. In some embodiments, the carbon is highly activated. In some embodiments, carbon is provided by carbonizing or pyrolyzing bituminous coal, tobacco material, softwood pulp, hardwood pulp, coconut shells, almond shells, grape seeds, walnut shells, macadamia shells, kapok fibers, cotton fibers, cotton linters, or any other material that is known to be suitable for the production of carbon particles useful in cigarette filters. Further examples of suitable carbonaceous materials include activated coconut hull based carbons available from Calgon Corp. as PCB 12x30 and GRC-11 12x30. Further examples

of suitable carbonaceous materials are coal based carbons available from Calgon Corp. as S-Sorb 12% Cu 12x30; BPL 12x30; CRC-11F 12x30; FCA 12x30, Cu, CrO_3; and SGL. Further examples of suitable carbonaceous materials are wood based carbons available from Westvaco as WV-B, SA-20 and BSA-20. Other carbonaceous materials are available from Calgon Corp. as HMC; ASC/GR-1 12x30 Cu, Ag, CrO_3; and SC II; and another carbonaceous material includes Witco Carbon No. 637. Other carbonaceous materials are described in U.S. patent application Ser. No. 569,325, filed Aug. 17, 1990; U.S. Pat. Nos. 4,771,795 to White, et al. and 5,027,837 to Clearman, et al.; and European Patent Application Nos. 236,922; 419,733 and 419,981, the contents of which are hereby incorporated by reference in their entireties.

[0145] In some embodiments, the carbon is granular, particulate, nanoparticulate, spherical, fibrous, or impregnated on paper. The terms "granular" and "particulate" are intended to encompass both non-spherical shaped particles and spherical particles, such as so-called "beaded carbon" as described in WO 03/059096 A1, which is hereby incorporated by reference in its entirety.

[0146] The size of the individual carbon particles or granules can vary, depending upon the design of the filter element. Typically, large size particles have a U.S. mesh size of about 6x16; medium size particles have a U.S. mesh size of about 12x30; and small size particles have U.S. mesh sizes of about 20x50 and 30x70. Carbonaceous materials also can have a monolithic form, a bonded granular form, a fibrous form, or an agglomerated form; or be combined with molecular sieves, alumina particles or ion exchange resin particles. In some embodiments, the size of the carbon particles is from about 4x6 mesh to about 100x300 mesh. In some embodiments, the size of the carbon particles is from about 4x8 mesh to about 50x200 mesh. In some embodiments, the size of the carbon particles is from about 6x12 mesh to about 40x100 mesh. In some embodiments, the size of the carbon particles is from about 8x16 mesh to about 30x70 mesh. In some embodiments, the size of the carbon particles is from about 4x6 mesh to about 12x40 mesh. In some embodiments, the size of the carbon particles is from about 12x40 mesh to about 40x100 mesh.

[0147] The amount of carbon present within the filter can also vary depending on the filter length, and is usually measured in mg of carbon per mm length of the filter. The amount of carbon used per length of the filter, or sections of the filter, depends on the desired

taste characteristics, amount of desired reduction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells and the configuration (e.g., Dalmatian, multiple sections, channel, cavity) of the filter. In some embodiments, the filter or a section of a multiple section filter comprises carbon in an amount ranging from about 1 mg/mm to about 40 mg/mm. In some embodiments, the filter or a section of a multiple section filter comprises carbon in an amount ranging from about 3 mg/mm to about 8 mg/mm. In some embodiments, the filter or a section of a multiple section filter comprises carbon in an amount ranging from about 5 mg/mm to about 10 mg/mm. In some embodiments, the filter or a section of a multiple section filter comprises carbon in an amount ranging from about 15 mg/mm to about 25 mg/mm. In some embodiments, the filter or a section of a multiple section filter comprises carbon in an amount ranging from about 18 mg/mm to about 22 mg/mm. In some embodiments, the filter or a section of a multiple section filter comprises carbon in an amount ranging from about 6 mg/mm to about 7 mg/mm. In some embodiments, the filter or a section of a multiple section filter comprises carbon in an amount of about 20 mg/mm. In some embodiments, the filter or a section of a multiple section filter comprises carbon in an amount ranging from about 3 mg/mm to about 40 mg/mm.

[0148] Beaded carbon differs from granulated carbon because granular carbon has a surface shape that is irregular from granule to granule, whereas beaded carbon has consistent spherical form from bead to bead. Maintenance of a uniform bead size at or about a pre-selected diameter promotes smooth flow and consistent packing of the beads during the manufacturing process. Any of a variety of beaded carbon suitable for cigarette filters known in the art can be used in the compositions and methods provided herein, for example, those beaded carbon materials discussed in U.S. Patent Application Publication Nos. 2006/0180164 to Paine III et al. and 2006/0201524 to Zhang et al., the contents of which are hereby incorporated by reference in their entireties.

[0149] In some embodiments, the carbon can be surface-modified to increase its mechanical strength. Any of a variety of surface-modified carbon known in the art can be used in the compositions and methods provided herein, for example, the surface modified carbon discussed in U.S. Patent Application Publication No. 2006/0144410 to Luan et al., the contents of which are hereby incorporated by reference in its entirety.

[0150] The carbon can be incorporated into the filter in a non-uniform manner. In some embodiments, granulated adsorbent, such as carbon, can be placed in a cavity within the filter element. However, the adsorbent could also be imbedded or dispersed within a section of filter material, such as a fibrous filter material (e.g., cellulose acetate tow), or incorporated into a paper, such as the carbon-containing gathered paper described in U.S. Pat. No. 5,360,023 to Blakley et al., the contents of which are hereby incorporated by reference in their entireties. In addition, an adsorbent material can be placed both in a cavity and imbedded in one or more of the sections of filter material, and the adsorbent material in the compartment and the adsorbent imbedded or dispersed in the filter material can be the same or different.

[0151] Certain carbonaceous materials can be impregnated with substances, such as transition metals (e.g., silver, gold, copper, platinum, palladium), potassium bicarbonate, tobacco extracts, polyethyleneimine, manganese dioxide, eugenol, and 4-ketononanoic acid. The carbon composition may also include one or more fillers, such as semolina. Grape seed extracts may also be incorporated into the filter element as a free radical scavenger.

[0152] In some embodiments, the carbon is coated and/or impregnated with a coating material. In some embodiments, the coating material is a metal. In some embodiments, the coating material is copper. Any of a variety of coated and/or impregnated carbon known in the art can be used in the compositions and methods provided herein, for example, the coated and/or impregnated carbon discussed in U.S. Patent Nos. 4,636,333 to Matkin and U.S. Patent No. 5,657,772 to Duke et al., the contents of which are hereby incorporated by reference in their entireties. One advantage of impregnating or coating a metal, such as copper, onto the carbon is that a significant reduction of negative biological materials in the cigarette smoke occurs.

Ion Exchange Resins

[0153] In addition to coating and/or impregnating the carbon particles, it is also contemplated that the ion-exchange resin, such as a weak base amine-containing resin, can also, or alternatively, be coated or impregnated with a coating material. In some embodiments, the ion-exchange resin is coated or impregnated with a metal, such as copper. In some embodiments, both carbon and ion-exchange resin are present in the filter, and

neither, one, or both are coated or impregnated with a coating material, such as a metal material, including for example, copper.

[0154] Ion-exchange resin, such as resin functionalized with primary amines, are nucleophiles capable of selectively removing reactive electrophiles from the vapor phase of cigarette smoke. The ion-exchange resin can comprise any polymer having active groups in the form of electrically charged sites capable of displacement upon interaction with ions of opposite charge. Typically, the ion-exchange resin comprises a polymer backbone, such as styrene, styrene-divinylbenzene copolymers, acrylates, methacrylates, phenol formaldehyde condensates, and epichlorohydrin amine condensates, and a plurality of electrically charged functional groups attached to the polymer backbone. The ion-exchange resin is preferably a weak base anion exchange resin or a strong base anion exchange resin. Exemplary resins include DIAION™ ion-exchange resins available from Mitsubishi Chemical Corp. (e.g., WA30 and DCA11) and DUOLITE™ ion-exchange resins available from Rohm and Haas (e.g., DUOLITE™ A7). Other ion-exchange resins with primary amine groups include PUROLITE™ A-143 and PUROLITE™ A-109, which is a primary amine functionalized polystyrene crosslinked with divinylbenzene in the form of macroporous spherical beads.

[0155] Generally, increasing the amount of the primary amines in a resin decreases the biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome. However, increasing the amount of primary means of the resin generally decreases the sensory perception, that is, the taste is impaired. Thus, although a resin such as A109 containing a high amount of primary amines performs well in removing toxicants, the sensory perception is not as great as resin containing mixtures of amines.

[0156] An ion exchange resin, such as a weak base amine-containing resin, may contain 1.3%-1.5% wt/wt, such as1.4% wt/wt of nitrogen atoms (N) in the form of amine functional groups. In one embodiment, the ion exchange at resin may contain 0.5%, 0.75%, 1.0%, 1.5%, 2.0% or more wt/wt of nitrogen atoms (N) in the form of amine functional groups.

[0157] A filter may contain 0mg – 100 mg such as10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg or more of an ion exchange resin.

[0158] The form of the ion-exchange resin can vary. In some embodiments, the ion-exchange resin is in solid particulate form. In some embodiments, the size of the ion-

exchange resin particles is from about 4x6 mesh to about 100x300 mesh. In some embodiments, the size of the ion-exchange resin particles is from about 4x8 mesh to about 50x200 mesh. In some embodiments, the size of the ion-exchange resin particles is from about 6x12 mesh to about 40x100 mesh. In some embodiments, the size of the ion-exchange resin particles is from about 8x16 mesh to about 30x70 mesh. In some embodiments, the size of the ion-exchange resin particles is from about 4x6 mesh to about 12x40 mesh. In some embodiments, the size of the ion-exchange resin particles is from about 12x40 mesh to about 40x100 mesh.

[0159] The ion-exchange resin can be selected taking into consideration that the contact conditions between the tobacco smoke and the adsorbent are dependent on a number of variables, including how strongly the smoker pulls the smoke through the filter as the cigarette is being smoked and how much of the tobacco rod has been consumed prior to each puff. Thus, it is advantageous that the ion-exchange resin have a surface area greater than about 35 m^2/g so that there is minimal diffusional resistance and the surface area functional sites are easily accessible. Materials with greater surface areas also demonstrate less noticeable performance decline if part of the surface is covered with a plasticizer.

[0160] The amount of ion-exchange resin in the filter can vary over a wide range. The amount of ion-exchange resin within the filter typically ranges from about 10 to about 250 mg, and a person having ordinary skill in the art would understand that any range within this broader range is contemplated. In some embodiments, the ion-exchange resin within the filter ranges from about 20 to about 200 mg. In some embodiments, the ion-exchange resin within the filter ranges from about 40 to about 150 mg. In some embodiments, the ion-exchange resin within the filter ranges from about 60 to about 100 mg.

[0161] Narrower ranges of smaller amounts of ion-exchange resin are also contemplated. In some embodiments, the ion-exchange resin within the filter ranges from about 10 to about 50 mg. In some embodiments, the ion-exchange resin within the filter ranges from about 20 to about 40 mg. In some embodiments, the ion-exchange resin within the filter ranges from about 35 to about 45 mg. In some embodiments, the ion-exchange resin within the filter is at least about 40 mg.

[0162] Narrower ranges of larger amounts of ion-exchange resin are also contemplated. In some embodiments, the ion-exchange resin within the filter ranges from

about 150 to about 250 mg. In some embodiments, the ion-exchange resin within the filter ranges from about 100 to about 200 mg. In some embodiments, the ion-exchange resin within the filter ranges from about 150 to about 200 mg.

[0163] The amount of ion-exchange resin present within the filter can also vary depending on the filter length, and is usually measured in mg of ion-exchange resin per mm length of the filter. The amount of ion-exchange resin used per length of the filter, or sections of the filter, depends on the desired taste characteristics, the amount of reduction biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in the human cells and the configuration (e.g., Dalmatian, multiple sections, channel, cavity) of the filter. In some embodiments, the filter or a section of a multiple section filter comprises ion-exchange resin in an amount ranging from about 3 mg/mm to about 40 mg/mm. In some embodiments, the filter or a section of a multiple section filter comprises ion-exchange resin in an amount ranging from about 3 mg/mm to about 8 mg/mm. In some embodiments, the filter or a section of a multiple section filter comprises ion-exchange resin in an amount ranging from about 5 mg/mm to about 10 mg/mm. In some embodiments, the filter or a section of a multiple section filter comprises ion-exchange resin in an amount ranging from about 15 mg/mm to about 25 mg/mm. In some embodiments, the filter or a section of a multiple section filter comprises ion-exchange resin in an amount ranging from about 18 mg/mm to about 22 mg/mm. In some embodiments, the filter or a section of a multiple section filter comprises ion-exchange resin in an amount ranging from about 6 mg/mm to about 7 mg/mm. In some embodiments, the filter or a section of a multiple section filter comprises ion-exchange resin in an amount of about 20 mg/mm. In some embodiments, the filter or a section of a multiple section filter comprises ion-exchange resin in an amount ranging from about 3 mg/mm to about 40 mg/mm.

[0164] The filter can also further comprise other adsorbent materials in addition to the carbon and/or ion-exchange resin. In some embodiments, the filter further comprises molecular sieve, clay, alumina, silica gel, and/or modified silica gel. The particle size of these additional adsorbents can vary over a wide range, including the mesh ranges referenced above to the carbon particles, and particularly from about 8x16 mesh to about 3x70 mesh.

[0165] Additional polymeric or plastic materials are also contemplated for inclusion into the filter. In some embodiments, the filter further comprises polymeric resin,

cellulose acetate tow plasticized using triacetin, polyethylene glycol, triethyleneglycol diacetate, and/or other plasticizer. Where the filter material comprises a plasticizer, such as triacetin or carbowax, the amount of plasticizer compared to the other filter material can be up to about a 1:1 ratio by weight. In some embodiments, the total amount of plasticizer is about 4 to about 20 percent by weight of the filter. In some embodiments, the total amount of plasticizer is about 6 to about 12 percent by weight of the filter.

[0166] Further adaptations can be made to the filters described herein. In some embodiments, the filter further comprises strands of tobacco. In some embodiments, the strands of tobacco comprise reconstituted tobacco. In some embodiments, the cigarette filter comprises micro-cavity fibers. Any of a variety of cigarette filters comprising micro-cavity fibers known in the art can be used in the compositions and methods provided herein, for example, those cigarette filters comprising micro-cavity fibers discussed in U.S. Patent Nos. 6,584,979 to Xue et al. and 6,907,885 to Xue et al., the contents of which are hereby incorporated by reference. Such a micro-cavity fiber may be impregnated with an adsorbent material, such as carbon and/or ion-exchange resin, such that the adsorbent material is inserted in the micro-cavity spaces of the fiber. The fibers contain open or semi-open micro cavities that include, but are not limited to, multilobal shaped fibers. One non-limiting example of such a fiber is Honeywell's TRIAD™ fiber having an internal void fractional volume from about 0.5 to about 0.6. These fibers are capable of mechanically or electrostatically entrapping fine particles inside the fiber micro-cavity channels. Multilobal shaped fibers containing end caps would be considered fibers with semi-open cavities. Multilobal fibers without the end caps could be considered fibers with open cavities. The fibers may be constructed from any material suitable for cigarette use, such as polyethylene, polypropylene, and polyesters. Othe r micro-cavity fibers having the same performance characteristics may be used in the practice of the present invention.

Filters and Reduced Risk Tobacco Can Be Combined to Create a Reduced-Risk Tobacco Product

[0167] Aspects of the invention concern cigarettes that are configured to biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome as compared to the amount of biological insult induced by a conventional or reference cigarette (e.g., 2R4F), in human cells, which contain the modified tobaccos and

filters discussed herein. In some embodiments, the reduced risk tobacco product contains a cut filler composition that comprises a cured tobacco blend having a tobacco cured to have a reduced level of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells contacted by smoke from said tobacco, relative to conventional tobacco. For example, the cut filler composition can comprise a cured tobacco blend having a Burley tobacco (e.g., LA Burley 21) cured (e.g., air cured) to have a reduced level of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells contacted by smoke from said Burley tobacco, relative to a conventional Burley tobacco, or a Flue-cured Burley tobacco. In some embodiments, the reduced risk tobacco product contains a cigarette wrapper with reduced ignition propensity that circumscribes said cut filler composition. In some embodiments, the reduced risk tobacco product contains a cigarette filter that comprises carbon, an ion-exchange resin, or both. The reduced-risk tobacco products designed in accordance with the teachings provided herein possess unexpected advantages over known tobacco products because the reduced-risk tobacco products provided herein are configured to reduce the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome, as compared to conventional tobacco products in human cells contacted by cigarette smoke. That is, the cut filler composition and the filter design selected to be incorporated into the reduced-risk tobacco products provided herein are selected according to their relative propensity to reduce the biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells contacted by cigarette smoke. In some embodiments, the benefits of the cut filler composition and the filter design selected to be incorporated into the reduced-risk tobacco products provided herein synergistically combine to provide even greater reduction biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells contacted by cigarette smoke.

[0168] The selection of the cut filler composition and the filter are described elsewhere herein, and the combination thereof can be analyzed for their relative propensity to reduce the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells contacted by cigarette smoke according to

the DNA DSB assays, clonogenic assays, and RNA transcriptome or proteome assays provided herein and otherwise known in the art.

[0169] The cigarettes provided herein also comprise wrappers, for example, cigarette paper. Cigarette wrappers are generally made of low-flash, self-extinguishing cigarette paper ranging from 10 Coresta units to 200 Coresta units, depending on the desired "tar" delivery and filter ventilation. Coresta units are a measure of paper permeability, which is modified either through paper porosity or the addition of electrostatic or mechanical holes, to reduce tobacco burnt during each puff and to alter burn rate and puff count. In an embodiment, the cigarette wrapper comprises cigarette paper ranging from about 10 to about 200 Coresta units. In an embodiment, the cigarette wrapper comprises cigarette paper ranging from about 26 to about 170 Coresta units. In an embodiment, the cigarette wrapper comprises cigarette paper ranging from about 50 to about 150 Coresta units. In an embodiment, the cigarette wrapper comprises cigarette paper ranging from about 80 to about 110 Coresta units. In an embodiment, the cigarette wrapper comprises cigarette paper having about 80 Coresta units. In an embodiment, the cigarette wrapper comprises cigarette paper having about 110 Coresta units.

[0170] The cigarette paper may be banded, non-banded, or provided with regions that are either banded or non-banded. In an embodiment, the cigarette paper is banded. In an embodiment, the cigarette paper is non-banded

[0171] Any useful cigarette paper additive known in the art may be added to the cigarette paper, including additives that modify the burn rate of the paper. Useful additives include citrates, such as sodium citrate, potassium citrate, magnesium citrate, citric acid, phosphates (including alkali metal phosphates, e.g., lithium, sodium, and potassium, magnesium phosphate, and ammonium phosphate), and mixtures thereof. In an embodiment, the cigarette paper comprises citrate. In an embodiment, the citrate is present in an amount from about 0.1% by weight to about 2% by weight. In an embodiment, the citrate is present in an amount from about 0.2% by weight to about 1.5% by weight. In an embodiment, the citrate is present in an amount of about 1% by weight.

[0172] One embodiment is directed to a method of making a filtered cigarette described herein comprising: preparing a blend of cured tobacco, wherein the blend comprises a non-transgenic Burley tobacco and a non-transgenic Flue-cured or Bright

tobacco, wherein the non-transgenic Burley tobacco in the blend is present in an amount of 85-92 or 45-70% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco, and the non-transgenic Flue-cured or Bright tobacco in the blend is present in an amount of 8-15 or 55-30% respectively by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco; determining at least one of total pore volume or pore volume distribution of an activated carbon; selecting an activated carbon having a total pore volume of 0.1 mL/g to 0.9 mL/g and/or having a certain percentage of the activated carbon having a pore volume distribution of 0.1 mL/g to 0.9 mL/g, wherein the percentage of carbon having the pore volume distribution is least about 50%; optionally measuring and/or selecting an activated carbon having an average pore diameter of 0.6 nm to 1.1 nm; incorporating the selected activated carbon into a cigarette filter; and generating a filtered cigarette that contains the blend of cured tobacco and the cigarette filter. In the method, the cigarette filter may further comprise a weak base amine-containing resin. The ratio of the carbon, such as activated carbon, to the weak base amine-containing resin in the filter in the method may be from about 1:1 to 1:4. In another embodiment, the ratio of a carbon to weak base amine-containing resin is about 1:1 to 1:3, 1:1 to 1:2, 1:2 to 1:4, 1:2 to 1:3, 1:3 to 1:4, or about 1:1, 1:2, 1:3 or 1:4, The weak base amine-containing resin used in the method may contain at least or equal to about 50% -100% of primary amine functional groups, such as 50-95%, 50-90%, 50-85%, 50-80%, 50-75%, 50-70%, 50-65%, 50-60%, 50-55%, 55-100%, 55-95%, 55-90%, 55-85%, 55-80%, 55-75%, 55-70%, 55-65%, 55-60%, 60-100%, 60-95%, 60-90%, 60-85%, 60-80%, 60-75%, 60-70%, 60-65%, 6 5-100%, 6 5-95%, 65-90%, 65-85%, 65-80%, 65-75%, 65-70%, 70-100%, 70-95%, 70-90%, 70-85%, 70-80%, 70-75%, 75-100%, 7 5-95%, 75-90%, 75-85%, 75-80%, 80-100%, 80-95%, 80-90%, 80-85%, 85-100%, 85-95%, 85-90%, 90-100%, 90-95%, 95-100%. The activated carbon used in the method may have an activity of 50-60.

[0173] In another embodiment, the method further comprises: generating mainstream smoke from the filtered cigarette; and measuring the presence or absence of a toxicant retained in the filter. In addition, the method may further comprise: generating mainstream smoke from the filtered cigarette; and measuring the appearance of the biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in

lung cells contacted with the mainstream smoke, a fraction of the mainstream smoke, or a smoke condensate.

[0174] Another aspect of the invention is directed to a kit comprising: a first cigarette comprising a first cigarette filter that comprises a carbon or a weak base amine-containing resin, or both; and a second cigarette comprising a second cigarette filter that comprises a carbon, a weak base amine-containing resin, or both, wherein the second cigarette filter is configured to retain a greater amount of a toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the first cigarette filter. In the kit, the second cigarette filter may comprise a greater amount of the carbon or the weak base amine-containing resin or both than the first cigarette filter.

[0175] Another aspect of the invention is directed to a method of reducing the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in cells that contact cigarette smoke comprising: advising a tobacco consumer of the need to reduce DNA DSBs in cells that contact cigarette smoke; and replacing a cigarette habitually consumed by the tobacco consumer with any of the cigarettes described above.

[0176] Another aspect of the invention is directed to a method of reducing biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in cells of a tobacco consumer comprising: identifying the tobacco consumer in need of a reduction biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in cells of the tobacco consumer; and replacing a cigarette habitually consumed by the identified tobacco consumer with any of the cigarettes described above. The identifying step may comprise analyzing the presence of a biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in cells of the tobacco consumer. This method may further comprise measuring the presence of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells in the cells of the tobacco consumer before and after providing any of the cigarettes described above. The cells may comprise lung cells, cheek cells, throat cells, or buccal cells.

[0177] In one embodiment, the habitually-consumed cigarette or the first cigarette is an American Blend comprising a ratio of approximately 40% Burley to 60% Flue-cured tobacco. In one embodiment, the first cigarette is comprises a blend of cured tobacco, wherein the blend comprises a non-transgenic Burley tobacco and a non-transgenic Flue-cured or Bright tobacco, wherein the non-transgenic Burley tobacco is present in an amount of about 45-70% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco; and the non-transgenic Flue-cured or Bright tobacco is present in an amount of about 55-30% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco.

[0178] In another embodiment, a second or subsequent cigarette comprises a blend of cured tobacco, wherein the blend comprises a non-transgenic Burley tobacco and a non-transgenic Flue-cured or Bright tobacco, wherein the non-transgenic Burley tobacco is present in an amount of about 85-92% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco; and the non-transgenic Flue-cured or Bright tobacco is present in an amount of about 8-15% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco.

Tobacco Products Can Be Modified Using the Filters and Reduced Risk Tobacco Provided Herein to Gradually Acclimate a Consumer to Using a Reduced-Risk Tobacco Product

[0179] A significant problem associated with the consumption of reduced risk tobacco products is that consumers associate these products with unacceptable sensory/perception properties. For example, reduced risk cigarettes often have disagreeable tastes and/or odors, which make it difficult for a consumer to accept these cigarettes and, especially, to convert from consuming a conventional cigarette to the reduced risk cigarette. Accordingly, many smokers revert to their usual brand of high risk cigarette after trying the reduced risk products. Thus, it is desirable to gradually adjust a smoker's taste and/or sensory/perception over time so as to convert or shift the smoker to the reduced risk cigarette. The conversion from a conventional cigarette to the reduced risk cigarette described herein can be accomplished by gradually adjusting the tobacco blend and/or filter composition so as

to slowly change the taste and sensory/perception of the consumer over time. By slowly evolving the taste and/or odor of a reduced risk cigarette by changing the filter composition, tobacco blend, or tobacco blend/filter composition, there is a reduced ability of a tobacco consumer to notice a sensory/perception difference between their usual high-risk cigarette and the reduced risk cigarette, which increases the likelihood that the tobacco consumer will continue to smoke the reduced risk product. A step-wise program to adjust the consumers taste to a reduced risk cigarette is contemplated.

[0180] In some embodiments, the filters and reduced risk tobaccos described herein are combined to provide a variety of reduced-risk cigarettes with varying blends, and amounts of carbon and low ion exchange resin.

[0181] A step-wise program or method, may comprise gradually reducing the exposure of a tobacco user to a toxicant that causes biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells comprising: identifying the tobacco user to receive a gradual reduction in exposure to a toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells; replacing a cigarette habitually consumed by the identified tobacco user with a first cigarette for a predetermined length of time, wherein the first cigarette comprises a first cigarette filter that comprises a carbon, a weak base amine-containing resin, or both; replacing the first cigarette with a second cigarette after the predetermined length of time, wherein the second cigarette comprises a second cigarette filter that comprises the carbon, the poly-amine containing resin, or both, wherein the second cigarette filter is configured to retain a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells in human cells than the first cigarette filter. The predetermined length of time may be about 3-6 weeks.

[0182] In another embodiment, this method may further comprise replacing the second cigarette after a second predetermined length of time with a third cigarette, wherein the third cigarette comprises a third cigarette filter that comprises the carbon, the weak base amine-containing resin, or both, wherein the third cigarette filter is capable of retaining a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death,

or perturbation of RNA transcriptome or proteome in human cells than the second cigarette filter.

[0183] Optionally, a third and/or fourth cigarette can be provided, wherein the third and/or fourth cigarette contains a third and/or fourth cigarette filter capable of retaining a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the second or third cigarette filter respectively.

[0184] In one embodiment, the filters, reduced risk tobaccos, and program described above are used in methods of reducing the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in cells that contact cigarette smoke (e.g., lung cells, cheek cells, throat cells or buccal cells), wherein a tobacco consumer is advised of the need to reduce the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in cells that contact cigarette smoke, by providing information or instructions to that effect.

[0185] In one embodiment, the advising can be performed by inclusion of a statement that the contents of the reduced-risk cigarette reduce the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in cells that contact cigarette smoke in a product label, such as a cigarette pack or carton.

[0186] In another embodiment, the filters, reduced risk tobaccos, and program above are combined in methods of reducing the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in cells of a tobacco consumer (e.g., lung cells, cheek cells, throat cells or buccal cells), wherein a tobacco consumer is identified as one in need of such reduction, and providing the reduced-risk cigarette comprising the filter/reduced risk tobacco combination to the tobacco consumer, either in the presence or absence of instructions. In one embodiment, the identification step comprises an analysis of the presence biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in cells of the tobacco consumer.

[0187] By gradually decreasing the amount of an agent that induces biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells and/or increasing the amount of ion exchange resin and/or carbon, the beneficial

effects of reduced toxicants can be obtained without a noticeable decrease in sensory/perception properties of the cigarette.

[0188] A kit based on these methods may comprise: a first cigarette comprising a first cigarette filter that comprises a carbon or a weak base amine-containing resin, or both; and a second cigarette comprising a second cigarette filter that comprises a carbon, a weak base amine-containing resin, or both, wherein the second cigarette filter is configured to retain a greater amount of a toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells in human cells than the first cigarette filter. In the kit, the second cigarette filter may comprise a greater amount of the carbon or the weak base amine-containing resin or both than the first cigarette filter. The kits may also include additional cigarettes as described below in more detail.

[0189] The cigarettes and kits described herein can also be used as part of a marketing program. Another aspect of the invention is directed to a method of marketing a cigarette that is configured to reduce the biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells comprising: replacing a cigarette habitually consumed by a tobacco consumer with a first cigarette comprising a first cigarette filter comprising a carbon and a weak base amine-containing resin for a predetermined length of time; replacing the first cigarette after the predetermined period of time with a second cigarette comprising a second cigarette filter configured to retain a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or RNA transcriptome or proteome in human cells than the first cigarette filter; and marketing the first and second cigarettes, wherein the first cigarette is introduced to a consumer prior to the second cigarette and the first cigarette is marketed for a time sufficient to adjust a tobacco consumer's taste prior to marketing the second cigarette. The time to adjust the tobacco consumer's taste may be less than 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months.

[0190] In another embodiment, the method of marketing the cigarette further comprises replacing the second cigarette with a third cigarette that comprises a third cigarette filter capable of retaining a greater amount of a toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells in human cells than the second cigarette filter. In another embodiment, the first cigarette, the

second cigarette and the third cigarette have substantially similar packaging. In further embodiment, the first cigarette, the second cigarette, and the third cigarette are sold under the same brand. In yet another embodiment, the first cigarette, the second cigarette, and the third cigarette have the same packaging.

[0191] The method of marketing described above they also comprise determining a tobacco consumer's acceptance of taste (e.g., by conducting a focus group or test analysis) of the first cigarette; providing a second cigarette to the tobacco consumer where the second cigarette comprises a second filter configured to retain a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the first cigarette filter, or wherein the second cigarette comprises a reduced risk tobacco and/or a second cigarette filter that comprises an ion-exchange resin and/or carbon, wherein the second cigarette is configured to reduce the biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells in human cells or deliver a reduced amount of an agent that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells relative to said first cigarette; determining the tobacco consumer's acceptance of taste (e.g., in a focus or test analysis) of the second cigarette; and marketing the first and second cigarettes, wherein the first cigarette is marketed for a time sufficient to adjust a tobacco consumer's taste before marketing the second cigarette. A determination of the time that is sufficient to adjust the tobacco consumer's taste or sensory/perception can be made in the focus or test group. Optionally, the reduced-risk tobacco and/or amount of ion-exchange resin or carbon that will be acceptable to a population of smokers (e.g., amount that will not substantially alter the sensory/perception properties of the cigarette) can be determined using standard market research surveys. Once an acceptable amount is determined, then a second cigarette having a second cigarette filter configured to retain a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells than the first cigarette filter, or a second cigarette comprising reduced-risk tobacco and/or more ion-exchange resin and/or carbon can be manufactured. Various tobaccos and/or amounts of ion-exchange resin or carbon, as described herein, can be used in the second cigarette, and again the optimal amounts of these components can be

determined using a market research survey, focus groups or test analysis. The same procedure can be followed to determine the filter that is configured to retain a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the third cigarette filter, or the type of tobacco and/or amount of ion-exchange resin and/or carbon to use in a third cigarette. A third cigarette is provided that comprises a third filter configured to retain a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or changes perturbation of RNA transcriptome or proteome in human cells than the second cigarette filter, or a third cigarette comprising tobacco and/or a third cigarette filter that comprises an ion-exchange resin and/or carbon, wherein the third cigarette has a third filter configured to retain a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the second cigarette filter; and/or is configured to deliver a reduced amount of an agent that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells relative to said second cigarette. By gradually modifying the filter that is configured to retain a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the second cigarette filter, for example, by modifying the tobacco and/or increasing the amount of ion exchange resin and/or carbon, the beneficial effects of reduced toxicants can be obtained without a noticeable decrease in sensory/perception properties of the cigarette, resulting in increased acceptance of a reduced risk cigarette by the tobacco user.

[0192] Accordingly, some embodiments include methods of gradually introducing the change in tobacco and filter composition to cigarette smokers so as to adjust a consumer's sensory perceptive behavior to the reduced risk cigarettes described herein. More such methods are accomplished by gradually increasing the capacity of a filter that is configured to retain a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the previous cigarette filter; for example, by increasing the amount of reduced-risk tobacco in the blend over time (e.g., an amount of tobacco containing a reduced number compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells) is provided in a tobacco

blend of a first cigarette that is marketed to consumers, after one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve months, a second cigarette having a second cigarette filter that is configured to retain a progressively greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the first cigarette filter, such as one having a tobacco blend with more tobacco having a reduced number of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells is provided in a cigarette to the consumer, optionally, a third, fourth, fifth or sixth cigarette can be provided to the consumer, wherein said successive generations of cigarettes are also provided after one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve months from the marketing of the previous generation of cigarette and said third, fourth, fifth or sixth cigarette has filter configured to retain a progressively greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the first cigarette filter, such as one that has progressively more tobacco having a reduced number of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells than the preceding cigarette.

[0193] In a similar fashion, more methods are accomplished using the cigarette designs described above by gradually increasing the ability of a filter that is configured to retain a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the first cigarette filter, for example by introducing more ion exchange resin and/or carbon to said tobacco consumer in successive cigarettes. That is, a first cigarette can have a first amount of carbon and/or resin in said filter and after a period of one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve months a second cigarette can be provided to said consumer and said second cigarette that has a second cigarette filter is configured to retain a progressively greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the first cigarette filter such as a filter that has more carbon and/or ion exchange resin than said first cigarette. Optionally, a third cigarette with a third cigarette filter that is configured to retain a progressively greater amount of the toxicant that induces biological insults such as

DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the second cigarette filter, such as a filter containing more carbon or ion exchange resin is provided to the tobacco consumer after one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve months of marketing the second cigarette and, additionally, a fourth, fifth or sixth cigarette that has a fourth, fifth, or sixth cigarette filter configured to retain a progressively greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the previous cigarette filter, such as by having progressively more carbon and/or ion exchange resin than the preceding cigarette, is marketed to the consumer one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve months after the preceding cigarette.

[0194] Further, the reduced-risk tobacco and/or the ion exchange resin can be gradually modified or increased in a stepwise fashion in combination. That is, a second cigarette having a filter configured to retain a greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the first cigarette filter, such as one in which the cigarette has an amount of tobacco having a reduced number of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells and an amount of ion exchange resin and/or carbon, can be provided in a first cigarette and after one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve months of smoking the first cigarette a second cigarette having more tobacco having a reduced number of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells and/or ion exchange resin and/or carbon and/or carbon pore volume can be provided to the consumer and after one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve months of smoking the second cigarette, optionally, a third cigarette having more tobacco with a reduced number of compounds cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells and/or ion exchange resin and/or carbon and/or carbon pore volume can be provided to said tobacco consumer after one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve months of smoking the second cigarette. Still further, a fourth, fifth, or sixth cigarette can be provided wherein each successive cigarette is provided after one, two, three, four, five, six,

seven, eight, nine, ten, eleven, or twelve months of smoking the preceding cigarette and each successive cigarette having a filter configured to retain a progressively greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the previous cigarette filter, such as a cigarette that has more tobacco having a reduced number of compounds that induce biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells and/or ion exchange resin and/or carbon and/or carbon pore volume in the cigarette over time. In this manner, an improved method of introducing a reduced risk cigarette to the market is provided, wherein a tobacco consumer's sensory/perception and taste behaviors are slowly adjusted while gradually modifying filter such that it is configured to retain a progressively greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the previous cigarette filter, such as by gradually increasing the presence of tobacco having a reduced number of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells and/or ion exchange resin and/or carbon and/or carbon pore volume in the cigarette after a period of time such that the consumer more readily receives the reduced risk cigarette and replaces the conventional cigarette used by the consumer prior to starting the program.

[0195] Additionally, the step-wise programs described above gradually reduce the presence of toxicants in the mainstream smoke of the cigarette over time because as the filter is configured to retain a progressively greater amount of the toxicant that induces biological insults such as DNA DSBs, cell death, or perturbation of RNA transcriptome or proteome in human cells than the previous cigarette filter, or as the tobacco having a reduced number of compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells and/or ion exchange resin and/or carbon and/or carbon pore volume are increased, the amount of toxicants present in the mainstream smoke of the cigarettes are decreased. In some embodiments it is preferred that substantially the same packaging is maintained during the step-wise programs above. In some contexts, by substantially the same packaging is meant that the cigarette brand is maintained with only slight variations in the labeling so as to maintain customer brand recognition while gradually changing the composition of the cigarettes, as described above.

[0196] In the embodiments described above in this section, the habitually-consumed cigarette or the first cigarette is an American Blend comprising a ratio of approximately 40% Burley to 60% Flue-cured tobacco. In one embodiment, the first cigarette comprises a blend of cured tobacco, wherein the blend comprises a non-transgenic Burley tobacco and a non-transgenic Flue-cured or Bright tobacco, wherein the non-transgenic Burley tobacco is present in an amount of about 45-70% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco; and the non-transgenic Flue-cured or Bright tobacco is present in an amount of about 55-30% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco.

[0197] In another embodiment, described above in the section, a second or subsequent cigarette comprises a blend of cured tobacco, wherein the blend comprises a non-transgenic Burley tobacco and a non-transgenic Flue-cured or Bright tobacco, wherein the non-transgenic Burley tobacco is present in an amount of about 85-92% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco; and the non-transgenic Flue-cured or Bright tobacco is present in an amount of about 8-15% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco.

[0198] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLES

Example I

Cigarette Design to Reduce Oxidative Stress and Subsequent Attenuation of Genotoxicity and Cytotoxicity Effects of Cigarette Smoke on Biological Systems

[0199] This example shows that blends having a high proportion of Burley tobacco to Flue-cured or Bright tobacco unexpectedly induce fewer DNA DSBs than blends having a low proportion Burley tobacco to Flue-cured or Bright tobacco. In addition, this example shows the unexpected result of less H2AX damage or cell death using a carbon filter with a cigarette containing a high proportion of Burley tobacco to Flue-cured or Bright tobacco. Further, this example shows a synergistic effect conflict of a filter containing a carbon and a weak base amine-containing resin.

[0200] Increased oxidative stress is a major mechanism by which cigarette smoke causes airway damage that can lead to a host of pathogenic conditions including asthma, pulmonary fibrosis, chronic obstructive pulmonary disease, and lung cancer. However, a detailed understanding of the specific molecular mechanisms that link oxidative stress with cigarette smoke-induced pathologies is still lacking. Increased knowledge in this area can be used developing new approaches to mitigating or reversing the damage caused by cigarette smoke either by chemopreventive strategies and/or reducing the toxicity of cigarettes. Cigarette smoke is a complex mixture of over 4000 different chemical components; some of which are highly oxidizing in nature such as reactive oxygen species (ROS), reactive nitrogen species (RNS) and substantial amounts of long lived and short lived radical species, primarily carbon and nitrogen centered. Despite efficient antioxidant defense mechanisms in the respiratory tract, the large amounts of short lived free radicals (such as •O2-, •NO, •OH-, etc), and more stable organic reactive species/oxidants (such as acrolein, epoxides, etc.) that exist in both the gas and particulate phases of cigarette smoke can transiently, and perhaps chronically, overwhelm the cell's steady-state antioxidant capacity. In fact, there is strong evidence that active smoking causes a marked imbalance in an individual's redox state and an overall increase in oxidative stress, especially in the respiratory tract. One possible result of this disruption to the lung's redox status is the induction of oxidized DNA, which can manifest as missing bases, altered or mismatched bases, deletions and insertions, strand breaks, intra and inter-strand cross-links, structural or numerical chromosomal aberrations, abnormal sister chromatid exchanges, and the formation of micronuclei, as well as protein/enzyme damage through covalent adduction, cross-linking, sulfide bond interactions and active site modifications including DNA repair proteins. Each of these defects can not only be genotoxic and cytotoxic, but can also play a fundamental role in tumorigenesis.

[0201] Comprehensive design of cigarettes is based on the individual design characteristics of traditional cigarette technology such as blend/blend component selection, filter design and filter additives as well as tobacco additives. Design characteristics are selected by combining information from chemical analysis and biological markers that support reduced oxidative stress in biological systems. The guiding principles for the selections are based on the predictability of specific results based on the Chem-Bio model associated with Lipid Peroxidation (LPO) induced oxidative stress and following the

chemical markers associated with LPO and biological markers connected to LPO induced oxidative stress.

A549 Cell Culture and Smoke Treatment

[0202] A549 cells are purchased from American Type Culture Collection (ATCC #CCL-185, Manassas, VA). The cells are cultured in Ham's F12K medium with 2mM L-glutamine adjusted to contain 1.5g/L sodium bicarbonate (ATCC, Manassas, VA) and supplemented with 10% fetal bovine serum (ATCC, Manassas, VA). Dual-chambered slides (Nunc Lab-Tek II) are seeded with 1 ml of 10^5 cells/ml cell suspension per chamber 48 hours before exposure. All incubations are at 37°C in a humidified atmosphere of 5% CO_2 in air. Cells are grown to 70% conFluency, at which time they are treated with smoke. The cell culture medium is replaced with 37°C Dulbecco's PBS (D-PBS) containing calcium and magnesium (Sigma, St. Louis, MO) for the smoke exposure. Slide chamber covers are removed and the slides were placed in a smoke exposure chamber (20.6cm x 6.7cm x 6.3cm - LxWxH). Smoke is generated from various cigarettes provided below under FTC smoking conditions using a KC 5 Port Smoker (KC Automation, Richmond, VA). The smoke is diluted by drawing it through a 250mL round-bottom flask prior to its reaching the exposure chamber. The time and distance that the smoke travels from the end of the cigarette to the exposure chamber is minimized by using the shortest lengths of tubing possible between the parts of the apparatus. Cigarettes are smoked to within 3 mm of the filter tip. Cells are exposed to smoke for up to 40 minutes. Mock-exposed (control) cells are treated under identical conditions as the exposed cells except for the absence of a cigarette in the smoking port. They are mock-exposed for 10 minutes. Following treatment or mock treatment, the D-PBS is aspirated and replaced with 1 ml per chamber of fresh culture medium at 37°C. The slides are placed in the 37°C, 5% CO_2 incubator and incubated for 15 minutes. Following incubation, the medium is aspirated and the slides are submerged in 50 ml conical tubes filled with 70% ethanol. The fixed slides are stored at 4°C prior to analysis.

Immunocytochemical detection of phosphorylated histone H2AX

[0203] Cells are treated with smoke (*i.e.*, A549) or smoke condensate (*i.e.*, NHBE) and fixed as described above, then rinsed twice in PBS and immersed in 0.2% Triton X-100 (Sigma) in a solution of 1% (w/v) bovine serum albumin (BSA; Sigma) in PBS for 30 min to suppress non-specific antibody binding. The cells are then incubated in 100 µl

volume of 1% BSA containing 1:200 dilution of anti-phosphorylated histone H2AX (γ-H2AX) rabbit polyclonal Ab. After overnight incubation at 4°C, the slides are washed twice with PBS and then incubated in 100 μl of 1:200 dilution of Alexa Fluor 488 goat anti-rabbit IgG (H+L) (Molecular Probes, Eugene, OR) for 45 min at room temperature in the dark. The cells are then counterstained with 1 μg/ml 4,6-diamidino-2-phenylindole (DAPI, Molecular Probes, Eugene, OR) in PBS for 5 min. Each experiment is performed with an IgG control in which cells are labeled only with secondary antibody, Alexa Fluor 488 goat anti-rabbit IgG (H+L) or FITC-conjugated F(ab')2 fragment of goat anti-mouse immunoglobulins, without primary antibody incubation to estimate the extent of nonspecific binding of the secondary antibody to the cells.

Measurement of cell fluorescence by Laser Scanning Cytometry

[0204] Cellular green (phosphorylated histone H2AX) fluorescence emission is measured using a Laser Scanning Cytometer (LSC; CompuCyte, Cambridge, MA), utilizing standard filter settings; fluorescence is excited with a 488-nm argon ion laser. The intensities of maximal pixel and integrated fluorescence are measured and recorded for each cell. At least 3,000 cells are measured per sample.

[0205] Phosphorylated histone H2AX may also be detected using commercial antibodies and Western blotting.

Clonogenicity assay

[0206] A clonogenic survival assay was used to study the ability of tobaccos and tobacco products to impact the proliferation of cells. The experiment involves four major steps: (1) inoculate cells into culture dishes and incubate for 24-48 hours; (2) upon reaching the logarithmic phase of growth, the treatment is applied; the treatment in this case is freshly prepared and diluted CS for increasing periods of time; (3) the cells are then allowed to recover for a set number of hours (up to 24), then the cells are trypsinized, replated at specific dilutions, and allowed to continue growing for 5-7 days; the number of cells used depends largely on the plating efficiency of the cell line and must be determined empirically prior to the experiment; and (4) at the conclusion of the experiment, the cells are fixed, stained, and counted. The primary measure is to count surviving colonies of 25-50 cells which is presented as the percentage of cells which survived the treatment. A graphical representation of survival versus exposure time to CS is then generated. The surviving

fraction is determined by dividing the number of colonies in the dish by the number of the colonies in the control (non-treated) dish.

[0207] A549 cells are exposed to smoke as described above. Following smoke exposure the medium is aspirated and the cells rinsed refed with 37°C BEGM and placed in a 37°C, 5% CO_2 humidified incubator for two to three hours. The cells are harvested by trypsinization with trypsin-EDTA (0.25% trypsin-0.38 mg/ml EDTA, Invitrogen). Cells are centrifuged at 260 x g for 8 min. Cell pellets are resuspended in 1 ml of Ham's F-12K medium, 10% FBS (complete medium) per pellet and counted. Cells are serially diluted so that the mock treated have ~65 cells per well and smoke treated have ~300 cells per well when seeded onto 96-well flat bottom tissue culture plates; one plate per condition. The plates are incubated for five days in a 37°C, 5% CO_2 humidified incubator. The colonies of cells are fixed with 5% formaldehyde/PBS and colored with 0.8% crystal violet solution for visualization. The colonies are counted with the aid of a macroscopic dissecting microscope. The cloning efficiency results are expressed in relation to the mock exposed cells.

Production of Cigarettes

[0208] Three test blends were produced with differing ratios of commercially available non-transgenic Flue-cured or Bright to non-transgenic Burley tobacco of about 90:10, 50:50 and 10:90. Each test blend contained approximately 63-66% tobacco lamina (Flue-cured and Burley combination), about 22 % expanded stem tobacco and about 12-15% Oriental tobacco as follows:

	10/90 Blend
Tob. Type:	% of Blend
Flue Cured	6.56%
Burley	59.07%
Oriental	12.37%
Cres Stem	22.00%
Total:	100.00%

	50/50 Blend
Tob. Type:	% of Blend
Flue Cured	31.53%
Burley	31.53%
Oriental	14.94%
Cres Stem	22.00%
Total:	100.00%

	90/10 Blend
Tob. Type:	% of Blend
Flue Cured	59.07%
Burley	6.56%
Oriental	12.37%
Cres Stem	22.00%
Total:	100.00%

[0209] Two cigarettes were produced per sample. These samples were designed to give similar tar and nicotine yields of about 9.0 mg tar and 0.8 mg nicotine. All cigarette

samples were machine made with cellulose acetate filters. Samples used for H2AX and Clonogenic assay comparisons included the machine made cellulose acetate filter of each blend type as control and hand modified samples of each blend type consisting of a plug space plug design where the space was filled with 40 mg, 60 mg, 80 mg, or 100 mg TA95 carbon.

[0210] The conditions included FTC Smoke from test cigarettes at 0% ventilation. Each of the three cigarette samples delivered approximately 100-120% more smoke than the 2RF4 control.

H2AX Damage Decreased with Increasing Amount of Activated Carbon

[0211] In the first experiment, cigarettes having five different filters were used, namely one control of the specific blend type either 90:10 flue cured/burley or 10:90 flue cured burley containing a conventional cellulose acetate filter, and four filters containing four different amounts of TA95 activated carbon, namely 40 mg, 60 mg, 80 mg, or 100 milligrams.

[0212] DSBs in relative fluorescence units compared to sham control were measured using the H2AX assay described with the following conditions: A549 cells were cultured in Ham's F12K medium with 2mM L-glutamine adjusted to contain 1.5g/L sodium bicarbonate (ATCC) and supplemented with 10% fetal bovine serum (ATCC). All incubations were at 37°C in a humidified atmosphere of 5% CO_2 in air.

[0213] Smoke treatment for A549 cells – dual-chambered slides (Nunc Lab-Tek II, VWR International, and West Chester, PA) were seeded with 1 ml of $5x10^4$ cells/ml cell suspension per chamber 48 hours before exposure and were typically at 70% confluency at the time of smoke treatment. The cell culture medium was replaced with 37°C Dulbecco's PBS (D-PBS) containing calcium and magnesium (BioSource, Rockville, MD) for the smoke exposure. Slide chamber covers were removed and the slides were placed in a smoke exposure chamber (20.6cm x 6.7cm x 6.3cm -LxWxH). Smoke was generated from 2R4F cigarettes (Kentucky Reference Cigarette, containing 8.9 mg "tar" and 0.75 mg nicotine per cigarette ref *(37)*] under Federal Trade Commission (FTC) smoking conditions (35 ± 0.3cc puff, one puff every 60 seconds, 2-second puff duration with none of the ventilation holes blocked) using a KC 5 Port Smoker (KC Automation, Richmond, VA). Cigarettes were smoked to within 3 mm of the filter tip. All cigarettes had been equilibrated at 23.9°C ±

1.1°C and 60% ± 2% relative humidity for a minimum of 24 hours and a maximum of 14 days. The smoke exposure chamber was designed to deliver smoke uniformly diluted with 5% CO_2 in air and passed through the cell exposure chamber at a constant flow rate of 500 cc/min. Briefly, each 35cc puff was first drawn into a 250cc chamber containing 5% CO_2 in air and mixed via a stir bar for 3 seconds. The diluted smoke was then passed over the cells at a flow of 500 ml/min. The standard smoke dilution used in most of our experiments was 35cc delivered over 58 seconds, and the intensity of exposure was varied by varying the length of time the cells spent in the exposure chamber. The time and distance that the smoke traveled from the end of the cigarette to the exposure chamber was minimized by using the shortest lengths of tubing possible between the parts of the apparatus.

[0214] Mock-exposed cells were treated under identical conditions as the exposed cells except for the absence of a cigarette in the smoking port, with cigarettes containing about 90:10 Flue-cured:Burley tobacco and filters comprising varying amounts of activated carbon (TA95). Following treatment or mock treatment, the D-PBS covering the cells was aspirated and replaced with 1 ml per chamber of fresh culture medium at 37°C. The cells were placed in the 37°C, 5% CO_2 incubator and incubated for various time points depending on the required experimental conditions.

[0215] The DSBs were measured that were induced by cigarette smoke from cigarette samples having one (1) conventional cellulose acetate (CA) filter, and four (4) filters comprising 40 mg, 60 mg, 80 mg, or 100 mg of TA95 activated carbon. Figure 2 illustrates the decrease in DSBs upon increasing amount of activated carbon present in the filter.

[0216] In a second experiment, under the same conditions results were obtained using cigarette samples containing 10:90 Flue-cured:Burley tobacco as shown in Figure 3. These results also illustrate a decrease in DSBs upon increasing amount of activated carbon present in the filter.

[0217] The H2AX damage was compared based on the blend of tobacco in the cigarette samples. The H2AX assay described above was used to determine the amount of DSBs as a percentage of DSBs induced by a cigarette smoke from a cigarette sample having a cellulose acetate control filter of a control cigarette sample. A cigarette sample comprising the 90:10 Flue-cured:Burley tobacco blend (left/blue) was compared to a cigarette sample

comprising 10:90 Flue-cured:Burley tobacco blend (right/red). The DSB percentages are illustrated for cigarette samples comprising four (4) filters containing 40 mg, 60 mg, 80 mg, or 100 mg of TA95 activated carbon. H2AX damage using the 10:90 Flue-cured:Burley tobacco blend was significantly lower than the 90:10 Flue-cured:Burley tobacco blend as shown in Figure 4.

[0218] A similar comparison was made using the clonogenic assay described above. Figure 5 illustrates the percentage of cell death using cigarette smoke from a control cigarette having a cellulose acetate filter, a cigarette sample containing a 90:10 Flue-cured:Burley tobacco blend (left/blue), and a cigarette sample containing a 10:90 Flue-cured:Burley tobacco blend (right/red). The cell death as a percentage of total cells is illustrated in Figure 5 for a cigarette sample comprising one (1) conventional cellulose acetate (CA) filter as the control and two (2) filters containing 40 mg and 100 mg of TA95 activated carbon. Surprisingly, in the cigarette sample having a filter comprising 100 mg of activated carbon (TA95), cell death was significantly lower for the 10:90 Flue-cured:Burley tobacco blend in comparison to the 90:10 Flue-cured:Burley tobacco blend.

Synergistic Effect of Activated Carbon and Ion Exchange Resin Filters

[0219] DSBs were measured using an H2AX assay for cigarette samples containing 50:50 Flue-cured:Burley tobacco and having three (3) filters containing 50 mg TA95 activated carbon, 50 mg A109 ion exchange resin, and 50 mg each of TA95 and A109. These results were compared with a control 2R4F cigarette sample containing a conventional cellulose acetate (CA) filter. Both TA95 activated carbon and A109 ion exchange resin mitigated the amount of DSBs when added independently to a cigarette filter at equivalent 50 mg loadings. The cigarette samples having filters containing 50 mg TA95 activated carbon or 50 mg A109 ion exchange resin did not result in a reduction in the DSBs in comparison to the control; however, the cigarette sample containing a filter having a combination of 50 mg each of TA95 and A109 illustrated a significant reduction in DSBs. Thus, when added to a cigarette filter in combination, activated carbon and ion exchange resin mitigate DSBs by a larger amount than would be predicted based on their independent loading analysis, thus illustrating an unexpected synergistic effect. Figure 8 shows that various ion exchange resins similarly decrease H2AX damage, and act synergistically when combined with activated carbon.

[0220] Similar synergistic effects were observed in the clonogenic analysis illustrated in Figure 7. Cloning efficiency of A549 cells (five days post-smoke exposure) based on a percentage of cell death was measured using a clonogenic assay described above for cigarette samples containing 50:50 Flue-cured:Burley tobacco blends. The results as a percentage of cell death is illustrated for cigarette samples containing one (1) control 2R4F conventional cellulose acetate (CA) filter, and three (3) filters containing 50 mg TA95 activated carbon, 50 mg A109 ion exchange resin, and 50 mg each of TA95 and A109. The cigarette sample having a filter comprising 50 mg TA95 activated carbon resulted in an approximately 10% decrease in cell death in comparison to the control. The cigarette sample having a filter comprising 50 mg A109 ion exchange resin resulted in a slightly higher percentage of cell death in comparison to the control. The cigarette sample having a filter comprising a combination of 50 mg each of TA95 and A109 resulted in a greater than 40% reduction in cell death in comparison to the control. Thus, A109 (50 mg) does not improve cloning efficiency by itself, but does when mixed with TA95, illustrating a synergistic effect.

Various Ion Exchange Resin Filters Behave Synergistically

[0221] Three different amine containing resins (A109, Duolite and CR20) in combination with 50 mg of TA95 carbon in a plug-space-plug-space-plug hand modified filter design on a 50:50 flue cured burley blend were compared. The resins were added to occupy the same volume as 50 mg of TA95 carbon (the resins are less dense than carbon so this volume for all the resins comprises less than 50 mg of any particular resin).

[0222] As can be seen in Figure 8 the three resin/carbon combinations tested reduce the number of DSBs as measured by H2AX at various efficacies, as described above, compared to a 100 mg TA95 control cigarette of the same blend.

[0223] Furthermore, Figure 8 shows that the effect of adding resin to the filter in concert with carbon is synergistic as the total volume of "filter additives" *e.g.,* resin plus carbon is equal to the total volume of 100 mg of TA95 carbon, but the observed effect to the H2AX is greater than additive.

Example II

[0224] Four cigarettes were compared using the H2AX assay described above. All four cigarettes are comprised of the same construction and contain the same 50:50 Flue-cured:Burley tobacco blend: 1) control cigarette filter consisted of cellulose acetate (CA)

2) a research 2R4F cigarette, 3) 50 mg sepiolite was added to a hand modified CA filter in a plug-space-plug design, and 4) 40 mg of Sepiolite + 10 mg A109 was added to the filter in a plug-space -plug design occupying the same volume as 50 mg of Sepiolite sample.

[0225] As shown in Figure 9, no benefit was observed toward reducing DSBs with sepiolite alone in comparison to the control cigarette, and little benefit was observed for the research 2R4F cigarette. Further, as shown in Figure 7, in a separate experiment, no benefit was observed toward reducing DSBs with 50 mg of A109 alone in comparison to the control 2R4F cigarette. A synergistic effect was observed upon mixing 40 mg of sepiolite with 10 mg of A109 resin with a significant reduction in DSBs, as shown by the reduction in Figure 9.

[0226] Sepiolite mixed with nominal amounts of weak-base primary amine resin has the benefits of reduced DSBs, reduced cost (of the resin) and less objectionable sensory/taste characteristics associated with weak-base primary amine resins.

Example III

[0227] This example describes several approaches that can be used in addition to or in lieu of the H2AX assay and the cloning efficiency assay to identify reduced risk prototype cigarettes, as compared to a reference cigarette or commercially available cigarettes (i.e., to determine whether one or more of the prototype blends, cigarettes or filters described herein produces a cigarette smoke that induces less of a biological insult than a reference cigarette, such as 2R4For a commercially available brand). The perturbation on the transcriptome and/or proteome of multiple types of lung cells, oral mucosal cells, and white blood cells (e.g., A549 cells, NHBE cells or cells obtained from a smoker) that have been contacted with mainstream smoke obtained from a cigarette containing a tobacco blend with or without a carbon and/or weak base-containing resin filter, manufactured as described herein, is compared to the impact on the transcriptome and/or proteome obtained from mainstream smoke generated from a reference cigarette with or without a filter (e.g., a cellulose acetate filter), such as the 2R4F or a commercially available cigarette. Optionally, the transcriptome and/or proteome of multiple types of lung cells that are mock-treated (i.e., subjected to the experimental protocol without contact with smoke (control)) can be analyzed to establish a transcriptome and/or proteome baseline. Suitable methods for transcriptome and proteome analysis are described in U.S. Pat. App. Pub. No. 2008/0052789, Yang et al.,

BMC Cancer. 2005 Jul 20;5:83; and Han et al., Am J Clin Oncol. 2008 Apr;31(2):133-9, all of which are hereby expressly incorporated by reference in their entireties.

[0228] Accordingly, in a first set of experiments, transcriptome and proteome measurements are obtained by generating mainstream smoke from reference cigarettes (e.g., the 2R4F cigarette or commercially available cigarettes) with or without a filter, contacting lung cells with mainstream smoke generated from the cigarettes, and conducting a microarray analysis. Cells isolated from a smoker that has smoked a commercially available brand (e.g., lung cells, cells of the oral mucosa, and white blood cells) can also be obtained by conventional techniques and analyzed, as described above. A mock or control experiment is, optionally, performed to determine the baseline transcriptome and proteome for the cells that have not been contacted with cigarette smoke. If the analysis is conducted on a human, lung cells, cells of the oral mucosa, and/or white blood cells obtained from a non-smoker can be analyzed. A prototype cigarette with or without a filter, manufactured as described herein, is then analyzed by contacting the cells with the mainstream smoke generated from the prototype cigarette, and conducting the same microarray analysis. In some experiments the same cells of the smoker analyzed above can be screened after a suitable period of smoking of the prototype cigarette (e.g., 1, 2, 3, 4, 5, 6, 7, or 8 weeks after transition from a commercial brand to the prototype cigarette). By comparing the results obtained from the microarray analysis generated from the reference cigarette to the prototype cigarette and/or the mock experiment, differences in the impact on the transcriptome and proteome between the reference cigarette and the prototype cigarette are revealed and a determination that the prototype cigarette perturbs the transcriptome and proteome less than the reference cigarette can be made.

[0229] The microarray analysis can be conducted by harvesting the cells after smoke treatment, extracting the total RNA, generating fluorescently labeled cDNA and applying the nucleic acids to a microarray chip according to the manufacturer's protocol. RNA integrity can be assessed using capillary gel electrophoresis. A commercially available genome-scale oligonucleotide library containing gene-specific 70-mer oligonucleotides representing 21,329 human genes can be used for microarray production (QIAGEN Inc., Valencia Calif.). Hybridization is performed and the microarray is scanned using a simultaneous dual color, 48-slide scanner (Agilent technologies). Fluorescent intensity can

be measured using commercially available software. To determine the differentially expressed genes, the analysis is confined to the set of genes that are expressed above background. Optionally, quantitative reverse transcriptase PCR can be performed for specific genes to determine differences in the level of expression. It should also be pointed out that the above techniques can be employed to determine not only changes in RNAs that encode proteins but also microRNAs, which have been linked to oxidative damage and/or carcinogenesis.

[0230] In a similar fashion, proteomic analysis can be conducted. Accordingly, proteins obtained from multiple types of cells (e.g., A549, NHBE, or isolated human cells from smokers, such as lung cells, cells of the oral mucosa, or white blood cells), which have been contacted with mainstream smoke generated from a prototype cigarette, manufactured as described herein, mainstream smoke generated from a reference cigarette (e.g., 2R4F or a commercially available brand) and, optionally, mock treated cells (control) are applied to metal affinity or weak cation exchange (WCX2) protein chips to generate mass spectra by surface-enhanced laser desorption/ionization (SELDI) time-of-flight mass spectrometry, or similar techniques that use differential hybridization to protein chips, separately or in combination with one or more types of mass spectrometric analyses (e.g., time-of-flight). As discussed previously, cells isolated from a smoker that has smoked a commercially available brand can be obtained by conventional techniques and analyzed. A mock or control experiment is, optionally, performed to determine the baseline proteome for the lung cells that have not been contacted with cigarette smoke. If the analysis is conducted on a human, lung cells, cells of the oral mucosa, and/or white blood cells obtained from a non-smoker can be analyzed. In some experiments, the same cells of the same smoker analyzed above can be screened after a suitable period of smoking of the prototype cigarette (e.g., 1, 2, 3, 4, 5, 6, 7, or 8 weeks after transition from a commercial brand to the prototype cigarette). Protein peak identification and clustering are made using Ciphergen Biomarker Wizard and Biomarker Pattern software. In more experiments, the isolated proteins are separated using HPLC or 2-D gel electrophoresis prior to application of SELDI (or similar techniques that use differential hybridization to protein chips, separately or in combination with one or more types of mass spectrometric analyses (e.g., time-of-flight)) and immunoassays can be performed to identify the presence, absence, and quantity of specific proteins.

[0231] Based on previous results, it is expected that the reference cigarette will show transcriptome and proteome changes in a substantial proportion of the genome (e.g., up to 10%) compared to the mock experiment. It is also expected that some of the genes in the cells contacted with smoke from the reference cigarette will be under-expressed, as compared to the mock experiment. Lung cells contacted with mainstream smoke generated from one or more of the prototype cigarettes described herein are expected to show transcriptome and proteome changes that resemble more closely the transcriptome and proteome profiles generated in the mock experiment, as compared to the reference cigarette. In this fashion, one may identify reduced risk cigarette prototypes prepared as described herein, as compared to reference cigarettes. As is understood by those of skill in the art, the above example can be performed using a variety of techniques in RNA, cDNA, and protein analysis, which provide the same information (i.e., whether the transcriptome and proteome of lung cells contacted with mainstream smoke generated from a prototype cigarette is perturbed less than the transcriptome and proteome of lung cells contacted with mainstream smoke generated from a reference cigarette).

Example IV

[0232] A filter comprising activated carbon in a range of pore volumes or pore sizes or both are tested in cigarette samples for cloning efficiency as measured by the clonogenic assay and the correlation of aldehydes with DNA DSBs as measured by the H2AX assay, or an essay that measures perturbation of RNA transcriptome or proteome, which assays are described above.

[0233] An activated carbon having a total pore volume of 0.1 mL/g to 0.9 mL/g and/or an activated carbon having a certain percentage of the activated carbon having a pore volume distribution of 0.1 mL/g to 0.9 mL/g, wherein the percentage of carbon having the pore volume distribution is least about 50%, and/or an activated carbon having an average pore diameter of 0.6 nm to 1.1 nm produce less fewer DNA DSBs than a conventional cellulose acetate cigarette filter, have greater cloning efficiency by showing a lower percentages of cell death, and/or have fewer perturbations of RNA transcriptome or proteome in human cells.

Example V

[0234] This example provides a stepwise program to gradually reduce the amount of toxicants in cigarette smoke, which contribute to biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells as compared to the amount of biological insult induced by another cigarette, such as a habitually consumed cigarette, allowing for a process to gain wider consumer acceptance of a reduced risk cigarette.

[0235] A product that is being sold to/used by consumers can be modified in a gradual or stepwise fashion in order to slowly alter the composition of the cigarette to a reduced risk cigarette without significantly changing the taste and/or consumer perception of the product in such a way that would lead to consumer rejection of the product. In one embodiment, the slowly altered composition would start from a tobacco blend and/or filter design that receives significant market acceptance, and would change to contain a tobacco and/or filter that is configured to reduce the biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human contacted by cigarette smoke, relative to the original cigarette product. One cigarette with reduced ability to cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells relative to the original cigarette product is a cigarette containing a higher amount of air-cured Burley tobacco relative to the original product. Another cigarette with reduced ability to cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome is a cigarette product containing a filter comprising carbon and/or resin, for example, in the ratio of 1:1 to 4:1, or a filter containing a carbon having the total pore volume described.. The degree to which the new, reduced-risk product possesses the property of reduced biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome in human cells can be assessed using the H2AX assay and/or the clonogenicity assay and/or the RNA transcriptome or proteome assay provided in Example I.

[0236] In one example, this step program includes a replacing a cigarette habitually slowed by a tobacco user with a cigarette having a filter comprising 40 mg of activated carbon. The 40 mg of carbon cigarette is then replaced after approximately 3-6 weeks, such as 30 days, with a cigarette having a filter comprising 60 mg of activated carbon. In another example, the 60 mg carbon cigarette is replaced after an additional approximately

3-6 weeks, such as an additional 30 days, with a cigarette having a filter comprising 80 mg of activated carbon. In another example, the 80 mg carbon cigarette is replaced after an additional approximately 3-6 weeks, such as an additional 30 days, with a cigarette having a filter comprising 100 mg of activated carbon.

[0237] The new reduced-risk product also is tested for consumer acceptance. A focus or test group of smokers, such as smokers of the original product, is provided with the new reduced-risk product. The test group is then examined by questionnaire inquiring about the comparison of the new reduced-risk product relative to the original product. Alternatively, the test group is monitored for its smoking habits while using the new reduced-risk product in comparison with the smoking habits while using the original products. Other known focus or test group assessments of new products also can be implemented. The results of the focus or test group assessment will indicate whether or not the new reduced-risk product is likely to be accepted by the general population of consumers of the original product. Test results indicative of a low level of consumer acceptance of the change will indicate that the product is to be redesigned to include a smaller and/or different change in the tobacco, and/or carbon and/or resin in the filter. Test results indicative of a high level of consumer acceptance of the change will indicate that the product can be released to a larger population, including an entire national population, to thereby provide a product with high consumer acceptance and a reduced risk of tobacco related disease.

[0238] The gradual modification of the product can be performed on a product having the identical packaging, or a packaging substantially similar and not obviously denoting a modification in the cigarette product design. In this way, a consumer accustomed to consuming a particular brand of cigarette product can experience a gradual decrease in the exposure to compounds that cause the induction of biological insult, for example, DSBs, cell death or perturbation of RNA transcriptome or proteome, and other toxicants without the consumer noting the modification of the product in such a way as to reduce consumer acceptance of the new reduced-risk product.

[0239] Various types of filter designs can be modified for use with the methods tobacco blends described in the application generally and in the Examples above. Below are some examples of such filters:

Example VI

[0240] A filter element having a multiple section filter is prepared wherein a filter plug at the mouth-end is made of cellulose acetate tow and is about 7 mm in length, a general adsorbent section adjacent to the filter plug consists of about 40 mg of activated coconut charcoal dispersed throughout plasticizer-treated cellulose acetate tow cut to deliver a section about 10 mm in length, and a selective adsorbent section adjacent to the general adsorbent section consists of about 40 mg of Duolite™ A7 dispersed throughout plasticizer-treated cellulose acetate tow cut to deliver a section about 10 mm in length. The filter is attached to a tobacco rod having a length of about 56.5 mm and containing about 617 mg of the tobacco blend described above.

Example VII

[0241] A filter element is prepared as in Example IV except that about 20 mg Duolite A7 is used in the selective adsorbent section instead of 40 mg.

Example VIII

[0242] A filter element is prepared as in Example IV except that about 60 mg Duolite A7 is used in the selective adsorbent section instead of 40 mg.

Example IX

[0243] A filter element having a length of about 27 mm for use with tobacco rod length of about 57 mm is prepared. The tipping material circumscribes the length of the filter element and extends about 4 mm down the length of the tobacco rod.

[0244] The filter element of the cigarette has a general multi-sectional configuration. Filter elements of this general type are available from Baumgartner Inc., Switzerland. The cigarette has a filter element comprising a 12 mm mouth-end cellulose acetate tow (2.5 denier per filament/35,000 total denier) segment with 7% triacetin, a 7 mm compartment filled with granular carbon available as G277 (85 carbon tetrachloride activity and size 20x50 mesh) from PICA, and an 8 mm cellulose acetate tow (8.0/32,000) tobacco-end segment with 7% triacetin.

[0245] A ring of laser perforations is provided around the periphery of each cigarette about 13 mm from the extreme mouth-end thereof. The perforations penetrate through the tipping paper and plug wrap, and can be provided using a Laboratory Laser Perforator from Hauni-Werk Korber & Co. KG.

Example X

[0246] Cigarettes are provided as described in Example VII, except the filter element comprises an 8 mm mouth-end end cellulose acetate tow (8.0/32,000) segment with 7% triacetin, a 7 mm compartment filled with granular carbon available as G277 (85 carbon tetrachloride activity and size 20x50 mesh) from PICA, and a 12 mm cellulose acetate tow (2.5/35,000) tobacco-end segment with 7% triacetin.

Example XI

[0247] A filter element having two plugs with diameter of 7.5 mm and lengths of 14.5 mm and 8 mm, respectively, is created. The weights of the first plug and the second plug are around 77 and 45 mg, respectively. Powders are loaded in the cylindrical space between these two plugs which have a dimension of about 7.5 mm OD×4.5 mm in length. Carbon powder is Pica coconut-based sample #99-2-3 with a median particle diameter of about 10 micrometers and an APS silica gel is used with specific surface treatment with median particle diameter of about 5 micrometers. The surface area of the APS silica gel is about 300 m^2/g and the surface area of the activated carbon is about 2000 m^2/g.

Example XII

[0248] A filter element according to Example IX is created, except the adsorbent powders are loaded in an amount of 25 mg in about 100 mg of semi-open micro-cavity fibers.

Example XIII

[0249] The following Table 1 illustrates several non-limiting embodiments of cigarettes that can be used with the methods described herein.

Table 1

Exemplary reduced risk cigarettes

Blend	Paper	Filter
Burley 21 LA, Flue Cured, Oriental, Conventional Burley	80 CU 1.0% citrate, banded	25 mm length with 7 mm cellulose acetate mouth end and 18 mm cellulose acetate with 40 mg carbon (Dalmatian) tobacco end cigarettes were made 60% dilution
Burley 21 LA, Flue Cured, Oriental, Conventional Burley	80 CU 1.0% citrate, banded	25 mm length with 7 mm cellulose acetate mouth end and 18 mm cellulose acetate with 60 mg carbon

Blend	Paper	Filter
		(Dalmatian) tobacco end cigarettes were made 60% dilution
Burley 21 LA, Flue Cured, Oriental, Conventional Burley	80 CU 1.0% citrate, banded	25 mm length with 7 mm cellulose acetate mouth end and 18 mm cellulose acetate with 80 mg carbon (Dalmatian) tobacco end cigarettes were made 60% dilution
Burley 21 LA, Flue Cured, Oriental, Conventional Burley	80 CU 1.0% citrate, banded	25 mm length with 7 mm cellulose acetate mouth end and 18 mm cellulose acetate with 108 mg carbon (Dalmatian) tobacco end cigarettes were made 60% dilution
Flue Cured, Oriental, Conventional Burley	26 CU 1.0% citrate	25 mm length with 7 mm cellulose acetate mouth end and 18 mm cellulose acetate with 108 mg carbon (Dalmatian) tobacco end
Flue Cured, Oriental, 21-41 Burley,	26 CU 1.0% citrate	25 mm length with 7 mm cellulose acetate mouth end and 18 mm cellulose acetate with 108 mg carbon (Dalmatian) tobacco end
Flue Cured, Oriental, Conventional Burley	110 CU 1.0% citrate	25 mm length with 7 mm cellulose acetate mouth end and 18 mm cellulose acetate with 108 mg carbon (Dalmatian) tobacco end
Flue Cured, Oriental, 21-41 Burley,	110 CU 1.0% citrate	25 mm length with 7 mm cellulose acetate mouth end and 18 mm cellulose acetate with 108 mg carbon (Dalmatian) tobacco end
Flue Cured, Oriental, Conventional Burley	26 CU 1.0% citrate	25 mm length with 8 mm cellulose acetate mouth end, 5 mm cavity with 95 mg carbon and 12 mm cellulose acetate with 68 mg carbon cigarettes were made at 26% dilution and 60% dilution
Flue Cured, Oriental, 21-41	26 CU 1.0% citrate	25 mm length with 8 mm

Blend	Paper	Filter
Burley,		cellulose acetate mouth end, 5 mm cavity with 95 mg carbon and 12 mm cellulose acetate with 68 mg carbon (Dalmatian) tobacco end cigarettes were made at 26% dilution and 60% dilution
Flue Cured, Oriental, Conventional Burley	110 CU 1.0% citrate	25 mm length with 8 mm cellulose acetate mouth end, 5 mm cavity with 95 mg carbon and 12 mm cellulose acetate with 68 mg carbon (Dalmatian) tobacco end
Flue Cured, Oriental, 21-41 Burley,	110 CU 1.0% citrate	25 mm length with 8 mm cellulose acetate mouth end, 5 mm cavity with 95 mg carbon and 12 mm cellulose acetate with 68 mg carbon (Dalmatian) tobacco end
Flue Cured, Oriental, Conventional Burley	170 CU 1.0% citrate	25 mm length with 8 mm cellulose acetate mouth end, 5 mm cavity with 95 mg carbon and 12 mm cellulose acetate with 68 mg carbon cigarettes were made at 0% dilution
Flue Cured, Oriental, 21-41 Burley,	170 CU 1.0% citrate	25 mm length with 8 mm cellulose acetate mouth end, 5 mm cavity with 95 mg carbon and 12 mm cellulose acetate with 68 mg carbon (Dalmatian) tobacco end cigarettes were made at 0% dilution
Flue Cured, Oriental, Conventional Burley	170 CU 1.0% citrate, non-banded	25 mm length with 8 mm cellulose acetate mouth end, 9 mm cavity containing carbon (124 mg) and 8 mm cellulose acetate with 40 mg carbon (Dalmatian) tobacco end
Flue Cured, Oriental, Conventional Burley	170 CU 1.0% citrate, non-banded	25 mm length with 9 mm cellulose acetate mouth end, 7 mm cavity containing carbon (94 mg) and 9 mm

Blend	Paper	Filter
Flue Cured, Oriental, Conventional Burley	170 CU 1.0% citrate, non-banded	cellulose acetate with cellulose acetate tobacco end 25 mm length with 9 mm cellulose acetate mouth end, 7 mm cavity containing carbon (137 mg) and 7 mm cellulose acetate with 40 mg carbon (Dalmatian) tobacco end
Flue Cured, Oriental, Conventional Burley	170 CU 1.0% citrate, non-banded	30 mm length with 9 mm cellulose acetate mouth end, 12 mm cavity containing carbon (179 mg) and 9 mm cellulose acetate with cellulose acetate tobacco end
Flue Cured, Oriental, Conventional Burley	170 CU 1.0% citrate, non-banded	25 mm length with 9 mm cellulose acetate mouth end, 12 mm cavity containing carbon (179 mg) and 9 mm cellulose acetate with 45 mg carbon (Dalmatian) tobacco end
Flue Cured, Oriental, Conventional Burley	170 CU 1.0% citrate, non-banded	25 mm length with 10 mm cellulose acetate mouth end, 10 mm cavity containing carbon (144 mg) and 10 mm cellulose acetate with cellulose acetate tobacco end
Flue Cured, Oriental, Conventional Burley	170 CU 1.0% citrate, non-banded	25 mm length with 10 mm cellulose acetate mouth end, 10 mm cavity containing carbon (144 mg) and 10 mm cellulose acetate with 50 mg carbon (Dalmatian) tobacco end

Blends contain 5-25% Oriental tobacco, 10-50% Burley (conventional, 21-LA or 21-41 or combinations thereof) and 10-50% Flue cured.

[0250] Various "reduced risk" technologies that are known in the art can be adapted for use with aspects of the invention. For example, the tobacco blends, low alkaloid/nicotine tobacco and blends containing this tobacco, filter technology, methods of cigarette design, methods of gradual adjustment of blends and filter components in successive generations of products to adjust consumer sensory perception over time as the

amount of toxicant consumed by the consumer is adjusted, and all other aspects described herein can be employed with or combined with known methodologies to develop improved cigarettes and methods of use thereof.

[0251] The following Table 2 provides a listing of references, each of which describe a technology that can be employed with or combined with the teachings provided herein. Each of the references in the Table 2 is incorporated herein by reference. In some embodiments, the following technologies known in the art can be modified by a teaching described herein:

Table 2

Technology	Patent Number	Priority Date	Author
Aminofunctional Silica Gel	US 20050161053	18-Mar-05	Xue, Lixin et al.
Aminofunctional Silica Gel	US 6907885	11-Feb-03	Xue, Lixin et al.
Aminofunctional Silica Gel	US 6911189	28-Jan-02	Koller et al.
Carbon, surface modified	WO 2006/070291 A2	29-Dec-05	Luan, Z. et al.
Carbon, surface modified	US 20060174899 A9	22-Dec-03	Luan, Z. et al.
Carbon bead	US 20060180164 A1	11-Apr-06	Paine, J. B. et al.
Carbon, monolithic pore size	US 20060201524 A1	19-Dec-06	Zhuang, et al.
Carbon sieve	US 20060207620 A1	15-Mar-05	Plunkett, S.B. et al.
Carbon with metal catalyst	US 20060231113 A1	13-Apr-05	Newberry, p. et al.
Carbon with Zeolite membrane	US 20060260626 A1	20-Dec-05	Luan, Z. et al.
Carbon with Tobacco beads for flavor	US 20070000505 A1	22-Feb-06	Zhuang, S. et al.
Carbon, monolithic pore size	US 20070000508 A1	29-Jun-05	Xue, Lixin et al.
Cellulose acetate microcavities	US 6779528	16-Apr-02	Xue, Lixin et al.
Carbon with metal catalyst	US 6789547	30-Oct-01	Paine, J. B. et al.
Carbon bead, cigarette	WO 03/059096A1	9-Jan-02	Paine, J. B. et al.
Carbon, flavored	WO 03/071886A1	22-Feb-02	Yang, Z. et al.
Carbon, Activated thread	WO 03/086116 A1	12-Apr-02	Xue, Lixin et al.
Resin, Polyaromatic Filter	WO 2004/019709A2	30-Aug-02	Xue, Lixin et al.
Carbon, Sorbent from resin	WO 2004/097309 A1	2-Apr-03	Zhuang, S. et al.
Carbon, Flavored	WO 2006/064371 A1	15-Dec-04	Banerjea, A. et al.
Carbon, apparatus for filling filter	WO 2006/072089 A1	30-Dec-05	Atwell, C.G. et al.
Carbon, surface modified	WO 2006/070291 A2	30-Dec-04	Luan, Z. et al.
Carbon, Activated with molecular sieve	WO 2006/072889 A1	5-Jan-05	Luan, Z. et al.
Molecular Sieve, flavored	WO 2006/085142 A2	22-Dec-04	Luan, Z. et al.
Carbon and molecular sieve	WO 2006/097852 A1	15-Mar-05	Plunkett, S.B. et al.
Carbon, templated	WO 2007/026253A2	29-Jun-05	Xue, Lixin et al.
Carbon, templated	WO 2007/031876 A2	29-Jun-05	Xue, Lixin et al.
Carbon beads	WO 2007/061094A2	13-Dec-05	Karles, G. et al.
Carbon and molecular sieve	US 20050133047	22-Dec-03	Fournier et al.
Molecular Sieve, ampiphilic	US 20050133048	22-Dec-03	Fournier et al.
Molecular sieve, zeolite	US 20050133049	22-Dec-03	Fournier et al.
Molecular sieve, thiol functionalized	US 20050133050	23-Dec-03	Fournier et al.
Alumina with carbon and zeolite particles	US 20050133051 A1	22-Dec-03	Luan, Z. et al.

Technology	Patent Number	Priority Date	Author
Aluminosilicate molecular sieve	US 20050133052	17-Nov-04	Fournier et al.
Molecular sieves, Copper impregnated	US 20050133053	22-Dec-03	Fournier et al.
Zeolite on cut filler	US 20050133054	22-Dec-03	Fournier et al.
Silica gel, surface modified	US 200502050102	27-Jan-05	Yang, Z. et al.
Carbon, surface modified	US 20060086366	19-Oct-05	Xue, Lixin et al.
Resin, Polyaromatic, Filter	US 6863074	30-Aug-02	Xue, Lixin et al.
Filter, Carbon & thread	WO 02/069745 A1	22-Feb-01	Jupe et al.
Filter, Shaped Microcavity	WO 03/047836A1	30-Nov-01	Xue, Lixin et al.
Filter manufacturing, central flavor unit	WO 03/082558	29-Mar-02	Lanier et al.
Filter, Adsorbents surface modified	WO 2004/010802A1	26-Jul-02	Thomas et al.
Silica powder with active protein or catalyst	WO 2004/047568A1	26-Nov-02	Desisto et al.
Filter, cavity filling apparatus	WO 2004/052129A2	9-Dec-02	Atwell, C.G. et al.
Shaped microcavity manufacturing process	WO 2004/062396A2	6-Jan-03	Xue, Lixin et al.
Filter, nanostructured fibril	WO 2005/039329A1	27-Oct-03	Saoud et al.
Process for surface modification of filter	WO 2006/051416A1	9-Nov-04	Karles, G. et al.
Filter, tobacco beads	WO 2006/090290 A1	24-Feb-05	Zhuang, et al.
Segmented rod, tobacco beads	WO 2006/100605 A1	21-Mar-05	Borgogron et al.
Filter, hollow fiber	WO 2007/054826 A2	4-Nov-05	Rasouli et al.
Filter, hollow fiber	US 20070074733 A1	4-Oct-05	Rasouli et al.
Flavor capsule, liquid release	WO 2007/060543 A2	15-Aug-05	Besso, C. et al.
Cigarette, coaxial tobacco rod in tobacco rod	WO 2007/069091 A2	12-Dec-05	Zhuang, et al.
Filter, bicarbonate treated fibers	WO 2007/069093 A2	13-Dec-05	Xue, Lixin et al.
Filter, bicarbonate treated fibers	US 20070181141 A1	11-Dec-05	Xue, Lixin et al.
Catalyst, CO reduction	WO 2007/083195 A2	13-Dec-05	Miser, D. et al.
Carbon or APS silica gel, electrostatic process	US 20050126481	27-Jan-05	Xue, Lixin et al.
Molecular sieve, functionalized SBA 15	US 20060130855	16-Sep-05	Luan, Z. et al.
Carbon, surface modified	US 20060144410	30-Dec-05	Luan, Z. et al.
Nanofiber, polysaccharide	US 20060264130	20-Dec-05	Karles, G. et al.
Sorbent with flavor additives	US 20060272662 A1	3-Feb-06	Jupe et al.
Filter, flavor capsule	US 20070012327	2-May-06	Karles, G. et al.
Cigarette, coaxial tobacco rod in tobacco rod	US 20070137667	11-Dec-06	Zhuang, et al.
Filter, carbon, catalyst and bypass channel	US 20070169785	22-Dec-06	Gedevanishvili et al.
Filter, concentric with carbon loaded core	US 5622190	15-Nov-94	Arterbery et al.
Filter, concentric with TOW	US 5746230	31-May-95	Arterbery et al.
Cigarette for electrical smoking system	US 5915387	31-Dec-96	Baggett et al.
APS Silical gel	US 6209547	29-Oct-98	Koller et al.
APS Silical gel	US 6595218	11-May-00	Koller et al.
Carbon, flavored	US 6761174	22-Feb-02	Jupe et al.
Filter, Shaped Microcavity	US 6762768	20-Apr-01	Xue, Lixin et al.
Cigarette, carbon or catalyst tip	US 6868855	1-Dec-03	Shafer et al.
Cigarette, carbon or catalyst tip	US 6874508	10-Apr-03	Shafer et al.

Technology	Patent Number	Priority Date	Author
Cigarette with thermally collapsible portion	US 6883523	14-Feb-03	Dante et al.
Microcavity, carbon or APS silica gel	US 6913784	5-Jul-05	Xue, Lixin et al.
Filter, electrostatic process carbon or APS silica gel	US 6919105	6-Jan-03	Xue, Lixin et al.
Recess filter with cavity	US 7243659	12-Jul-00	Lecoultre et al.
Cavity with central filter element	WO 03/049560A1	11-Dec-01	Lauenstein, M. et al.
Filter, monolithic segments	WO 2004/086888	2-Apr-03	Zhuang, et al.
Filter, Carbon & thread	WO 2006/082525A1	4-Apr-05	Jupe et al.
Filter, flavor capsule	WO 2006/082529 A2	4-Feb-05	Karles, G. et al.
Filter, flavor capsule	WO 2006/117697 A1	3-May-05	Karles, G. et al.
Filter, flavor encapsulated in PVA	WO 2007/03694A2	30-Sep-05	Becker, U. et al.
Filter, peel n sniff, microcapsules flavor	WO 2007/05210A2	1-Nov-05	Wyss-Peters et al.
Cigarette, hollow tube with flavorant	WO 2007/099408A2	21-Dec-05	Xue, Lixin et al.
Flavored carbon	US 2040226569A1	16-Jun-04	Yang, Z. et al.
flavor thread process	US 20050255978 A1	6-Jul-05	Lanier et al.
Flavor Capsule	US 20060112964	9-Nov-05	Jupe et al.
Flavor Capsule	US 20060174901	10-Aug-06	Karles, G. et al.
Flavor Capsule	US 2006/0174901 A1	4-Feb-05	Karles, G. et al.
Nanoparticles, Fe on filler for CO reduction	WO 03/086115 A1	12-Apr-02	Li, P. et al.
Nanoparticle catalyst , CO reduction	WO 03/020058 A1	31-Aug-01	Li, P. et al.
Nanoparticles, Fe on filler for CO reduction	WO 03/086112 A1	8-Apr-02	Li, P. et al.
Nanoparticles, on Filler	WO 2004/041008 A1	4-Nov-02	Li, P. et al.
Nanoparticle, Cu and Ce	WO 2004/052520 A2	9-Dec-02	Deevi et al.
Nanoparticle, paper oxyhydroxide	WO 2005/039326 A2	27-Oct-03	Rasouli et al.
Nanoparticle, paper, filler, oxyhydroxide CO, NO reduction	WO 2005/039327 A2	27-Oct-03	Reddy, B. et al.
Nanoparticle, paper, filler, oxyhydroxide CO, NO reduction	WO 2005/039328 A2	27-Oct-03	Rabiei, S. et al.
Nanoparticle, preparation	WO 2005/039331 A2	28-Oct-03	Rabiei, S. et al.
Nanoparticle, paper, filler, oxyhydroxide CO, NO reduction	WO 2005/039332 A2	27-Oct-03	Reddy, B. et al.
Nanoparticle, paper, filler, Silver	WO 2005/122805 A2	16-Jun-04	Rangaraj, S. et al.
Nanoparticle,Au-Ce CO reduction	WO 2006/046145 A2	25-Oct-04	Pillai et al.
nanoparticles, taste	WO 2007/010405 A1	20-May-05	Rasouli et al.
Metal Nanowires, filler selective reduction	WO 2007/072231A2	20-Dec-05	Luan, Z. et al.
Encapsulated catalyst, CO, NO reduction	WO 2007/083245 A2	17-Jan-06	Gedevanishvili et al.
Catalyst, CO reduction, filter	WO 2007/096785 A2	27-Feb-06	Deevi et al.
Metal oxide, filler	US 20050126583	25-Oct-04	Rabiei, S. et al.
Nanoparticle, paper oxyhydroxide	US 20050155616	25-Oct-04	Rasouli et al.
Metal Oxide, CO reduction	US 20050166935	25-Oct-04	Reddy, B. et al.
Metal catalyst on tobacco powder, CO reduction	US 20060196517	30-Jan-06	Gedevanishvili et al.
Copper oxide, zinc oxide, cerium oxide CO reduction	US 20060289024	9-Mar-06	Deevi et al.
Copper oxide catalyst method of	US 20070014711	9-Mar-06	Deevi et al.

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formation			
Au-Ce catalyst for CO reduction	US 20070056601 A1	15-Jun-06	Pillai et al.
Metal Nanowires, filler selective reduction	US 2007016361 A1	13-Dec-06	Luan, Z. et al.
Catalyst, CO reduction, filter, paper	US 20070163612 A1	11-Dec-06	Miser, D. et al.
Catalyst, CO reduction, filter, paper	US 20070204870 A1	26-Jan-07	Deevi et al.
Nanoparticle, paper, filler, oxyhydroxide CO, NO reduction	US 6769437	8-Apr-02	Hajaligol et al.
Nanoparticle on silica support	US 6848450	7-Feb-01	Lilly et al.
Nanostructrured Fibril	WO 2005/039329A1	10/27/2003	Saoud, K. et al.
Nanoparticle, paper, filler, Silver	WO 2005/122805 A3	16-Jun-04	Rangaraj, S. et al.
Nanoparticle, paper, filler, oxyhydroxide CO, NO reduction	WO 2004/110184 A2	13-Jun-03	Li, P. et al.
Nanoparticle, CO reduction	WO 2004/110190A2	13-Jun-03	Koller et al.
Preparation of intermetallics	WO 2004/110591A2	13-Jun-03	Deevi et al.
Nanoparticle, Palladium	WO 2006/046153 A1	25-Oct-04	Deevi et al.
Cu-Ce catalyst for CO reduction	US 20050069023 A1	28-Sep-04	Deevi et al.
Nanoparticle catalyst, CO reduction	US 20050109356 A1	25-Oct-04	Reddy, B. et al.
Synthesis of nanoscale particles	US 20050166934	10/25/2004	Deevi et al.
Paper with ferrite catalyst	US 20050211259 A1	25-Oct-04	Gedevanishvili et al.
Synthesis of nanoscale particles	US 20050263162	25-Oct-04	Rabiei, S. et al.
Synthesis of nanoscale particles	US 2005063163	25-Oct-04	Yadav, R. et al.
Synthesis of nanoscale particles	US 2005063164	11-Mar-05	Reddy, B. et al.
Synthesis of nanoscale particles	US 20060032510	25-Oct-04	Deevi et al.
Synthesis of nanoscale particles	US 20060185685	15-Dec-05	Pithawalla et al.
Cigarette, electrical smoking system	US 20060185687	15-Dec-07	Hearn, J.R. et al.
Nanoparticles, oxidant catalyst	US 20070113862	21-Jun-06	Li, P. et al.
Nanoparticle, Manganese dioxide reduction of CO and NO	US 6782892	30-Aug-02	Li, P. et al.
Cu-Ce catalyst for CO reduction	US 6857431	9-Dec-02	Deevi et al.
Nanoparticle, iron catalyst reducing butadiene in smoke	US 7004993	13-Jun-03	Pithawalla et al.
Catalyst, CO reduction	US 7011096	31-Aug-01	Li, P. et al.
Catalyst, CO reduction	US 7017585	4-Nov-02	Li, P. et al.
Catalyst, CO reduction	US 7152609	13-Jun-03	Li, P. et al.
Nanoatalyst, CO reduction aluminosilicate	US 7165553	13-Jun-03	Luan, Z. et al.
Catalyst, CO & NO reduction	US 7168431	7-Apr-03	Li, P. et al.
Nanoparticle, oxyhydroxide	US 7228862	23-Feb-04	Hajaligol et al.
Catalyst, CO reduction	US 7243658	13-Jun-03	Deevi et al.
Nanoatalyst, CO reduction aluminosilicate	WO 2004/110183 A2	13-Jun-03	Luan, Z. et al.
Nanocatalyst, CO reduction aluminosilicate	WO 2004/110183 A3	14-Jun-03	Luan, Z. et al.
Nanoparticle catalyst , CO reduction, Fe	WO 2004/110189A3	13-Jun-03	Rasouli et al.
Paper with printed catalyst	WO 2005/002370A2	13-Jun-03	Li, P. et al.
Nanoparticle catalyst , CO reduction, Fe	WO 2005/039330 A1	27-Oct-03	Gedevanishvili et al.
Gravure print banded cigarette paper	WO 2007/020532 A1	15-Aug-05	Sherwood, T. et al.

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Smoke attenuator	US 20040079380	10-Apr-03	Shafer et al.
Process for making ammonium magnesium phosphate	US 20050279475	27-Jan-05	Fournier et al.
Corrugated paper with catalyst	US 20070169786	12/19/2006	Li, P. et al.
hydromagnesite, magnesium hydroxide paper	US 5927288	24-Mar-97	Bensalem, et al.
hydromagnesite, magnesium hydroxide paper	US 5979461	24-Mar-97	Bensalem, et al.
Paper heat degradable filler	WO 2003/043450	15-Nov-01	Hajaligol et al.
APS Silical gel	WO 00/25610	11-May-00	Koller et al.
APS Silical gel	WO 00/25611	29-Oct-98	Koller et al.
Ammonium magnesium phosphate cigarette paper	WO 01/08514	28-Jul-99	Fournier et al.
Process for making ammonium magnesium phosphate	US 7052581	25-Jul-02	Winterson, W.D. et al.
Cigarette with porous heat transfer tube	WO 2004/073427 A1	14-Feb-03	Dante et al.
Ammonia release in lit end	WO 2006/046150	25-Oct-04	Fournier et al.
Electrically heated cigarette	WO 2006/048774 A1	2-Nov-04	Newman, D.J. et al.
Perforated capsule with flavor in filter	WO 2006/051422 A1	10-Nov-04	Jupe et al.
Electrically heated cigarette	WO 2006/067627 A1	22-Dec-04	Hearn, J.R. et al.
Electrically heated cigarette	WO 2007/039794 A2	30-Sep-05	Braunshteyn et al.
Tobacco extraction process	WO 2007/052159 A2	29-Jul-05	Howell et al.
Metal amine complexes ammonia release	WO 2007/083196 A2	13-Dec-05	Xue, Lixin et al.
Cigarette, hollow tube with sorbent filter	WO 2007/093852A2	29-Dec-05	Olegario, R. et al.
Filter, carbon and bypass channel	WO 2007/09610 A2	29-Dec-05	Gedevanishvili et al.
Nanostructrured Fibril	US 20050121047 A1	25-Oct-04	Saoud, K. et al.
Smoke attenuator	US 20050178399 A1	21-Mar-05	Shafer et al.
Mesoporous sieve with flavor	US 20060130861 A1	22-Dec-04	Luan, Z. et al.
Encapsulated flavor with carbon filter	US 20060144412 A1	30-Dec-04	Mishra, M.K. et al.
segmented rod	US 20060254607 A1	20-Mar-06	Borgogron et al.
Electrically heated cigarette	US 20070074734 A1	30-Sep-05	Braunshteyn et al.
Electrically heated cigarette	US 20070102013 1	28-Sep-06	Adams, J.M. et al.
Cigarette, hollow tube with flavorant	US 20070181140 A1	9-Dec-06	Xue, Lixin et al.
Cigarette, hollow tube with heat sink	US 20070186945 A1	20-Dec-06	Olegario, R. et al.
Electrically heated cigarette	US 5988176	27-Aug-97	Baggett et al.
Electrically heated cigarette	US 6026820	12-Sep-97	Baggett et al.
Reduced sidestream , sleeve	US 6311694	2-Jul-99	Nichols, W.A. et al.
Reduced sidestream , sleeve	US 6367481	4-Feb-00	Nichols, W.A. et al.
Tobacco with inorganic oxide, phosphate, carbon or mixture for reduced combustion temperature	US 6637439	31-Aug-01	Hajaligol et al.
Smoke attenuator	US 6701936	11-May-01	Shafer et al.
Cigarette paper with heat degradable materials	US 6817365	15-Nov-01	Hajaligol et al.
Reduced sidestream	US 6823073	21-Feb-02	Nichols, W.A. et al.
Aerosol generating device	US 6923179	5-Sep-03	Gupta et.al.
Cigarette paper with heat degradable materials	WO 03/043450 A1	15-Nov-01	Hajaligol et al.

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cigarette with adsorbent in tip	WO 2004/039181	13-May-04	Thomas et al.
Tobacco with inorganic oxide, phosphate, carbon or mixture for reduced combustion temperature	WO 03/020059 a1	31-Aug-01	Hajaligol et al.
Reduction of TSNA's	WO 03/022081 A1	7-Sep-01	Krauss, M.R. et al.
Pd salts to reduce PaH's	WO 2004/110185 A2	17-Jun-04	Olegario, R. et al.
Shredded paper with catalyst, CO reduction	WO 2004/11088 A2	13-Jun-03	Gedevanishvili et al.
Nanoparticle, paper oxyhydroxide, CO reduction	WO 2004/110189 A3	13-Jun-03	Rasouli et al.
Phenol removal green tobacco, smoke phenol reduction	WO 2005/099493A2	14-Apr-04	Mcgrath, T.E. et al.
Cytotoxicity reduction addition of glycerine and Pd, Mg or Ca	WO 2006/046149A2	25-Oct-04	Olegario, R. et al.
Phenol removal green tobacco, smoke phenol reduction	WO 2006/059229A1	1-Dec-04	McGrath, T.E. et al.
Addition of tar, diluents to tobacco (long chain hydrocarbons)	WO 2007/012980	1-Jun-05	Lipowicz et al.
TSNA reduction	US 20050121046	5/12/2004	Hempfling, W. et al.
Phenol removal green tobacco, smoke phenol reduction	US 20050279374	13-Apr-05	McGrath, T.E. et al.
TSNA reduction, light treatment	US 20060016125	20-Jun-05	Krauss, M.R. et al.
Cytotoxicity reduction addition of glycerine and Pd, Mg or Ca	US 20060086367 A1	19-Oct-05	Li, S. et al.
Ammonia release	US 20060090708 A1	21-Oct-05	Fournier et al.
PaH removal tobacco, extraction	US 20060162733	30-Jul-05	McGrath, T.E. et al.
Nanostructrured Fibril	US 20060174903 A9	25-Oct-04	Saoud, K. et al.
TSNA reduction throught genetic modification	US 20060260014A1	15-Dec-05	Li, Q. et al.
Addition of tar, diluents to tobacco (long chain hydrocarbons)	US 20060283469 A1	1-Jun-06	Lipowicz et al.
Reconstituted tobacco, flavored	US 20070084476A1	17-Oct-06	Yang, S. et al.
Ammonia release	US 20070137666 A1	11-Dec-06	Xue, Lixin et al.
Elevated tobacco temperature during processing	US 5533528	30-Dec-93	Wallace er. al.
Vanillin release additive	US 5538018	5-Apr-95	Chan et al.
TSNA reduction	US 6564808	11-Aug-00	Hempfling, W. et al.
TSNA reduction	US 6755200	17-Nov-00	Hempfling, W. et al.
cigarette with adsorbent in tip	US 6860273	25-Oct-02	Thomas et al.
Paper, ammoniated filler	US 7216652	14-May-02	Fournier et al.
cigarette with adsorbent in tip	WO 2004/039181 A1	25-Oct-02	Thomas et al.
PaH removal tobacco, extraction	US 2006/0162733	30-Nov-05	McGrath, T.E. et al.
Carbon, Propylene glycol treated	WO 2007/033272 A1	9/14/2005	Coleman, W. M. et al.
Carbon, Propylene glycol treated	US 20070056600	9/14/2005	Coleman, W. M. et al.
Filter, multicompartment	WO 2005/032287A2	9/29/2004	Crooks et al.
Filter, ion exchange resin, flavor capsule	US 2005066980	9/30/2005	Crooks et al.
Filter, ion exchange resin, flavor capsule	US 2005066981	9/30/2005	Crooks et al.
Filter, carbon, flavor capsule	US 7240678	9/30/2003	Crooks et al.

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Filter, ion exchange resin, flavor capsule	US 2005066982	9/30/2005	Clark, M.A. et al.
Filter, icarbon, flavor capsule	US 7237558	9/30/2003	Clark, M.A. et al.
Filter, ion exchange resin, flavor capsule	US 2005066983	9/30/2005	Clark, M.A. et al.
Filter, ion exchange resin, flavor capsule	US 2005066984	9/30/2005	Crooks et al.
Filter, flavor capsule	US 20060272663 A1	8/14/2006	Dube, M. et al.
Aerosol generating system	US 20070023056 A1	8/1/2005	Cantrell et al.
Filter, Carbon paper or molecular sieve paper	US 5404890	6/11/1993	Gentry et al.
Filter, Carbon paper or molecular sieve paper	US 5568814	6/22/1994	Gentry et al.
Ultrafine metal catalyst on solid support	WO 2005/055747 A2	12/9/2003	Banerjee et al.
Ultrafine metal catalyst on carbon fuel element	WO 2006/002001 A2	6/15/2004	Banerjee et al.
Catalyst between tobacco rod and filter	US 7231923	7/13/2004	Adiga et al.
catalyst on carbon fuel element	WO 90/10394	3/16/1989	Shannon, M.D. et al.
Ultrafine particles in cigarette	US 20040173229	3/5/2003	Crooks et al.
Ultrafine particles in cigarette	US 20050121044	12/9/2003	Banerjee et al.
Banded paper ethyl cellulose or EVA	US 2003013186 A1	11/25/2002	Ashcraft, C.K. et al.
Banded paper ethyl cellulose or EVA	US 20050016556 A1	10/15/2003	Ashcraft, C.K. et al.
Banded paper ethyl cellulose, EVA, nitrocellulose, CAP, PVA	US 20050241659 A1	7/7/2005	Ashcraft, C.K. et al.
Banded paper EVA, PVA	US 20050241660 A1	7/7/2005	Ashcraft, C.K. et al.
Banded paper	US 20060005847 A1	9/13/2005	Chapman, P.S. et al.
Banded paper	US 20060011207 A1	9/13/2005	Chapman, P.S. et al.
Banded paper, flavored bands	US 20060124146 A1	2/10/2006	Stokes, C.S. et al.
Online banding of paper	US 20060207617 A1	5/19/2006	Seymour, S.K. et al.
2 papers, outer high Coresta, with flavors	US 20070051383 A1	8/1/2006	Woods, D.D.
Paper with pattern of materials added	US 20070084475 A1	10/14/2005	Oglesby, R.L. et al.
Paper with pattern of materials added	US 20070137668 A1	12/15/2005	Borschke, A.J. et al.
Cigarette with multiple wrappers	US 20070157940	1/6/2006	Mua, J.P.
Filter, flavor capsule	WO 2005/000044 A2	6/23/2003	Dube, M. et al.
Aerosol generating system	WO 2005/032285 A1	9/30/2003	Nestor, T. et al.
Aerosol generating system	WO 2007/015735 A1	8/1/2005	Cantrell et al.
Smokeless tobacco	WO 2007/037962 A1	9/22/2005	Holton, D.K.
Double wrapper cigarette	WO 2007/082145 A1	1/6/2006	Mua, J.P.
Aerosol generating system	US 20050066985 A1	9/30/2003	Borschke, A.J. et al.
Aerosol generating system	US 20050066906 A1	9/30/2003	Nestor, T. et al.
segmented filter manufacturing process	US 20070068540 A1	9/23/2005	Thomas, T. F. et al.
Carbon in web with menthol	US 20070204869 A1	9/28/2005	Sampson, J.R. et al.
Aerosol generating system	WO 99/63844 A1	6/10/1998	White, J.L. et al.
Tobacco with increased amino acids	WO 00/22946	10/22/1998	Shu, K. et al.

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Tobacco flavoring	WO 00/28840	11/18/1998	Coleman, W. M. et al.
Oriental tobacco heat treatment	WO 2004/041006 A1	10/21/2003	Lawson, J.W. et al.
Oriental tobacco	US 20040084056	10/31/2005	Lawson, J.W. et al.
Reconstituted tobacco with Tocopherol	US 20040255965	6/17/2003	Perfetti, T. et al.
Reconstituted tobacco with Tocopherol	US 20070107743	1/17/2007	Perfetti, T. et al.
Phenol formaldehyde beads/ carbonized	US 20070191571 A1	2/14/2006	Sink et al.
Phenol formaldehyde beads/ carbonized	US 20070191572 A1	11/8/2006	Tustin et al.
Phenol formaldehyde beads/ carbonized	US 20070191573 A1	11/8/2006	Sink et al.
Phenol formaldehyde beads/ carbonized	US 20070191575 A1	11/10/2006	Sumner et al.
Phenol formaldehyde beads/ carbonized	US 20070207917 A1	11/8/2006	Sink et al.
Silica resin	US 20020166564	3/26/2002	Sung
Carbon Filter	US 20030106562	2/13/2002	Chatterjee
smoking cessation, additive	US 20030178038	5/14/2002	Yamashita, H.
smoking cessation, additive	US 6748956	5/14/2002	Yamashita, H.
Metal phthalocyanine in filter	US 20060289023	8/24/2006	Von Borstel et al.
Metal phthalocyanine in filter	US 7104265	3/17/2004	Von Borstel et al.
Metal phthalocyanine in filter	US 20040173227	3/17/2004	Von Borstel et al.
Filter, carbon paper	US 5732718	3/31/1998	Douglas et al.
Filter, carbon, ferrous sulfate, l-ascorbic acid	US 7228861	1/30/2004	Atobe et al.
Filter, capsule with flavor	US 7249605	10/10/2003	Macadam et al.
Filter, multisegment	WO 95/07633	3/23/1995	Peters, G.
Filter, gas phase removal segment	WO 2006/081931	1/16/2006	Peters, G.
Metal oxide catalyst, selective reduction	WO 2005/012045	9/15/2004	Finlay, W. et al.
Metal Oxide cigarette paper LIP	WO 03/088771 A1	4/22/2002	Finlay, W. et al.
Metal Oxide cigarette paper LIP	WO 2003/088771	4/22/2002	Woodley, J.H.
Cigarette, low tar to nicotine ratio	US 20020034220 A1	2/15/2007	Pandolfino J. et al.
LIP cigarette paper	US 6837248	3/28/2003	Zawadzki, M.A. et al.
Detachable double filter cigarette	WO 01/28368 a1	9/6/2000	Grzonka, H. et al.
LIP cigarette paper	WO 02/0453513 A	6/28/2000	Dyakanov A.J. et al.
Filter rod made of tea	WO 2003/056946	10/30/2002	Feng, T.C. et al.
Smokeless cigarette	WO 2001/039619	6/17/2001	Shimizu, T. et al.
RIP paper	US 5878753	5/11/1997	Peterson, R.M. et al.
RIP paper	US 5878754	3/10/1997	Peterson, R.M. et al.
RIP paper	US 6568403 B2	6/15/2001	Hampl, V. et al.
RIP paper	US 6725867 B2	11/13/2001	Peterson, R.M. et al.
RIP paper	US 6779530 B2	1/23/2002	Kraker, T.A. et al.
Filler with Foaming agent	US 20070062550	10/20/2004	John, E.D. et al.

[0252] All references cited herein are incorporated herein by reference in their entirety. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

[0253] The term "comprising" as used herein is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps.

[0254] All numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the preferred embodiments. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches.

[0255] The above description discloses several methods and materials of the preferred embodiments. This invention is susceptible to modifications in the methods and materials, as well as alterations in the fabrication methods and equipment. Such modifications will become apparent to those skilled in the art from a consideration of this disclosure or practice of the invention disclosed herein. Consequently, it is not intended that this invention be limited to the specific embodiments disclosed herein, but that it cover all modifications and alternatives coming within the true scope and spirit of the invention as embodied in the attached claims.

WHAT IS CLAIMED IS:

1. A cigarette comprising a blend of cured tobacco, wherein said blend comprises a non-transgenic Burley tobacco and a non-transgenic Flue-cured or Bright tobacco, wherein the non-transgenic Burley tobacco is present in an amount of 45-70% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco, and the non-transgenic Flue-cured or Bright tobacco is present in an amount of 55-30% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco.

2. A cigarette comprising a blend of cured tobacco, wherein said blend comprises a non-transgenic Burley tobacco and a non-transgenic Flue-cured or Bright tobacco, wherein the non-transgenic Burley tobacco is present in an amount of 85-92% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco, and the non-transgenic Flue-cured or Bright tobacco is present in an amount of 8-15% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco.

3. The cigarette of Claim 1 or 2 wherein the blend further comprises expanded stem tobacco or Oriental tobacco.

4. The cigarette of any one of Claims 1-3, wherein the cigarette is configured to produce a mainstream smoke that generates fewer DNA double strand breaks (DNA DSBs) in lung cells than the mainstream smoke from a 2R4F reference cigarette under the same smoking conditions.

5. The cigarette of any one of Claims 1-4, further comprising a filter that comprises a carbon.

6. The cigarette of any one of Claims 1-5, further comprising a filter that comprises or wherein the filter comprises a weak base amine-containing resin.

7. The cigarette of any one of Claims 1-6, wherein said non-transgenic Burley tobacco is a low alkaloid variety.

8. The cigarette of any one of Claims 1-7, wherein said non-transgenic Flue-cured or Bright tobacco is a low alkaloid variety.

9. The cigarette of Claim 8, wherein the non-transgenic Burley tobacco is LA Burley 21.

10. The cigarette of any one of Claims 1-9, wherein said non-transgenic Burley tobacco is present in the amount of about 50% by weight, and wherein the non-transgenic Flue-cured or Bright tobacco is present in the amount of about 50% by weight.

11. The cigarette of any one of Claims 1-10, wherein said filter comprises a carbon that has a total pore volume of 0.1 mL/g to 0.9 mL/g.

12. The cigarette of any one of Claims 5-11, wherein the ratio of said carbon to said weak base amine-containing resin in said filter is from about 1:1 to 4:1.

13. The cigarette of any one of Claims 4-12, wherein said filter comprises about 30-100 mg of the carbon.

14. The cigarette of any one of Claims 5-13, wherein said filter comprises about 10-50 mg of the weak base amine-containing resin.

15. The cigarette of Claim 13, wherein said a weak base amine-containing resin contains at least or equal to about 1.3-1.5% nitrogen atoms (N) in the form of amine functional groups.

16. The cigarette of any one of Claims 4-15, wherein said carbon is an activated carbon having an activity of 50-60.

17. The cigarette of any one of Claims 4-16, wherein said filter further comprises sepiolite.

18. The cigarette of Claim 16 or 17, wherein said activated carbon is TA95.

19. The cigarette of any one of Claims 1-18, wherein said filter is a tripartite design comprising three compartments for containing each of cellulose acetate, the carbon, or the weak base amine-containing resin, wherein the carbon is downstream from the weak base amine-containing resin.

20. A cigarette filter comprising a carbon that has a total pore volume of from about 0.1 mL/g to about 0.9 mL/g in the cigarette filter.

21. The cigarette filter of Claim 20, wherein said total pore volume is from about0.4 mL/g to about 0.7 mL/g.

22. The cigarette filter of any one of Claims 20-21, further comprising a weak base amine-containing resin.

23. The cigarette filter of any one of Claims 20-22, wherein the ratio of said carbon to said weak base amine-containing resin in said filter is from about 1:1 to 4:1.

24. The cigarette filter of any one of Claims 20-23, wherein said cigarette filter comprises about 30-100 mg of carbon.

25. The cigarette filter of any one of Claims 20-24, wherein said filter comprises about 10-50 mg of weak base amine-containing resin.

26. The cigarette filter of any one of Claims 22-25, wherein said weak base amine-containing resin contains at least or equal to about1.3-1.5 nitrogen atoms (N) in the form of amine functional groups.

27. The cigarette filter of any one of Claims 20-26, wherein said carbon is an activated carbon having an activity of 50-60.

28. The cigarette filter of any one of Claims 20-27, wherein said filter further comprises sepiolite.

29. The cigarette filter of Claim 27 or 28, wherein said activated carbon is TA95.

30. The cigarette filter of any one of Claims 20-29, wherein said filter is a tripartite design comprising three compartments for containing each of cellulose acetate, the carbon, or weak base amine-containing resin, wherein the carbon is downstream from the weak base amine-containing resin.

31. A cigarette comprising the cigarette filter of any one of Claims 20-30.

32. A method of making a filtered cigarette comprising:

preparing a blend of cured tobacco, wherein said blend comprises a non-transgenic Burley tobacco and a non-transgenic Flue-cured or Bright tobacco, wherein the non-transgenic Burley tobacco in said blend is present in an amount of 30-55% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco, and the non-transgenic Flue-cured or Bright tobacco in said blend is present in an amount of 45-70% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco;

determining at least one of total pore volume or pore volume distribution;

selecting an activated carbon having a total pore volume of 0.1 mL/g to 0.9 mL/g and/or having a certain percentage of the activated carbon having a pore

volume distribution of 0.1 mL/g to 0.9 mL/g, wherein the percentage of carbon having the pore volume distribution is least about 50%;

optionally measuring and/or selecting an activated carbon having an average pore diameter of 0.6 nm to 1.1 nm;

incorporating said selected activated carbon into a cigarette filter; and

generating a filtered cigarette that contains said blend of cured tobacco and said cigarette filter.

33. A method of making a filtered cigarette comprising:

preparing a blend of cured tobacco, wherein said blend comprises a non-transgenic Burley tobacco and a non-transgenic Flue-cured or Bright tobacco, wherein the non-transgenic Burley tobacco in said blend is present in an amount of 85-92% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco, and the non-transgenic Flue-cured or Bright tobacco in said blend is present in an amount of 8-15% by weight based on the combined weight of the non-transgenic Burley tobacco and the non-transgenic Flue-cured or Bright tobacco;

determining at least one of total pore volume or pore volume distribution of an activated carbon;

selecting an activated carbon having a total pore volume of 0.1 mL/g to 0.9 mL/g and/or having a certain percentage of the activated carbon having a pore volume distribution of 0.1 mL/g to 0.9 mL/g, wherein the percentage of carbon having the pore volume distribution is least about 50%;

optionally selecting an activated carbon having an average pore diameter of 0.6 nm to 1.1 nm;

incorporating said selected activated carbon into a cigarette filter; and

generating a filtered cigarette that contains said blend of cured tobacco and said cigarette filter.

34. The method of Claim 32 or 33, wherein said cigarette filter further comprises a weak base amine-containing resin.

35. The method of any one of Claims 32-34, wherein the ratio of said activated carbon to said weak base amine-containing resin in said filter is from about 1:1 to 4:1.

36. The method of Claim 34-35, wherein said weak base amine-containing resin contains at least or equal to about1.3-1.5% nitrogen atoms (N) in the form of amine functional groups.

37. The method of any one of Claims 32-36, wherein said activated carbon has an activity of 50-60.

38. The method of any one of Claims 32-37 further comprising:

generating mainstream smoke from said filtered cigarette; and

measuring the presence or absence of a toxicant retained in the filter.

39. The method of any one of Claims 32-37 further comprising:

generating mainstream smoke from said filtered cigarette; and

measuring the appearance of DNA double strand breaks (DNA DSBs) in lung cells contacted with said mainstream smoke, a fraction of said mainstream smoke, or a smoke condensate.

40. A kit comprising:

a first cigarette comprising a first cigarette filter that comprises a carbon or a weak base amine-containing resin, or both; and

a second cigarette comprising a second cigarette filter that comprises a carbon, a weak base amine-containing resin, or both, wherein said second cigarette filter is configured to retain a greater amount of a toxicant that induces DNA DSBs in human cells than said first cigarette filter.

41. The kit of Claim 40, wherein said second cigarette filter comprises a greater amount of the carbon or the weak base amine-containing resin or both than the first cigarette filter.

42. A method of reducing the induction of DNA double strand breaks (DNA DSBs) in cells that contact cigarette smoke comprising:

advising a tobacco consumer of the need to reduce DNA DSBs in cells that contact cigarette smoke; and

replacing a cigarette habitually consumed by said tobacco consumer with the cigarette of any one of Claims 1-19 and Claim 31.

43. A method of reducing the induction of DNA double strand breaks (DNA DSBs) in cells of a tobacco consumer comprising:

identifying the tobacco consumer in need of a reduction in DNA DSBs in cells of said tobacco consumer; and

replacing a cigarette habitually consumed by said identified tobacco consumer with the cigarette of any one of Claims 1-19 and Claim 31.

44. The method of Claim 43, wherein said identifying step comprises analyzing the presence of DNA DSBs in cells of said tobacco consumer.

45. The method of Claim 43 or 44, further comprising measuring the presence of DNA DSBs in said cells of said tobacco consumer before and after providing the cigarette of any one of Claims 1-19 and Claim 31.

46. The method of any one of Claims 44-45, wherein said cells are lung cells, cheek cells, throat cells, or buccal cells.

47. A method of gradually reducing the exposure of a tobacco user to a toxicant that induces DNA double strand breaks (DNA DSBs) comprising:

identifying the tobacco user to receive a gradual reduction in exposure to a toxicant that induces DNA DSBs in human cells;

replacing a cigarette habitually consumed by said identified tobacco user with a first cigarette for a predetermined length of time, wherein said first cigarette comprises a first cigarette filter that comprises a carbon, a weak base amine-containing resin, or both;

replacing the first cigarette with a second cigarette after said predetermined length of time, wherein said second cigarette comprises a second cigarette filter that comprises the carbon, the poly-amine containing resin, or both, wherein said second cigarette filter is configured to retain a greater amount of the toxicant that induces DNA DSBs in human cells than said first cigarette filter.

48. The method of Claim 47, wherein the predetermined length of time is about 3-6 weeks.

49. The method of Claim 47 or 48 further comprising:

replacing the second cigarette after a second predetermined length of time with a third cigarette,

wherein said third cigarette comprises a third cigarette filter that comprises the carbon, the weak base amine-containing resin, or both, wherein said third cigarette

filter is capable of retaining a greater amount of the toxicant that induces DNA DSBs in human cells than said second cigarette filter.

50. A method of marketing a cigarette that is configured to reduce the induction of DNA double strand breaks (DNA DSBs) in human cells comprising

replacing a cigarette habitually consumed by a tobacco consumer with a first cigarette comprising a first cigarette filter comprising a carbon and a weak base amine-containing resin for a predetermined length of time;

replacing the first cigarette after the predetermined period of time with a second cigarette comprising a second cigarette filter configured to retain a greater amount of the toxicant that induces DNA DSBs in human cells than said first cigarette filter; and

marketing said first and second cigarettes, wherein said first cigarette is introduced to a consumer prior to said second cigarette and said first cigarette is marketed for a time sufficient to adjust a tobacco consumer's taste prior to marketing said second cigarette.

51. The method of Claim 50, wherein said time to adjust the tobacco consumer's taste is less than 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months.

52. The method of Claim 50 or 51, further comprising replacing the second cigarette with a third cigarette that comprises a third cigarette filter capable of retaining a greater amount of a toxicant that induces DNA DSBs in human cells than said second cigarette filter.

53. The method of any one of Claims 51-52, wherein said first cigarette, said second cigarette and said third cigarette have substantially similar packaging.

54. The method of any one of Claims 51-53, wherein said first cigarette, said second cigarette, and said third cigarette are sold under the same brand.

55. The method of any one of Claims 51-54, wherein said first cigarette, said second cigarette, and said third cigarette have the same packaging.

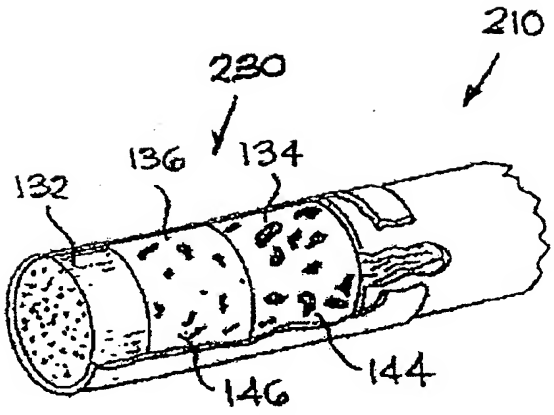


FIG. 1

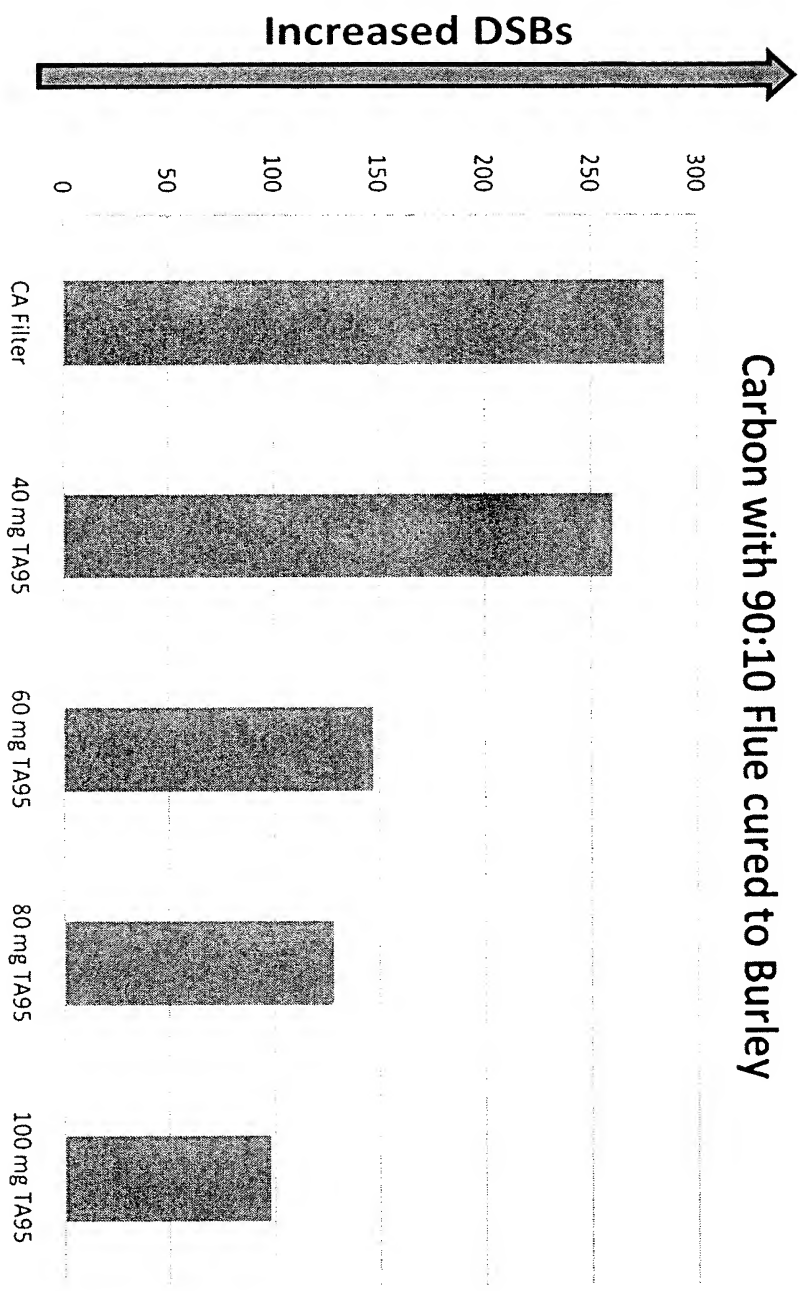

Increased DSBs
0
50
100
150
200
250
300
CA Filter
40 mg TA95
60 mg TA95
80 mg TA95
100 mg TA95
Carbon with 90:10 Flue cured to Burley

FIGURE 2

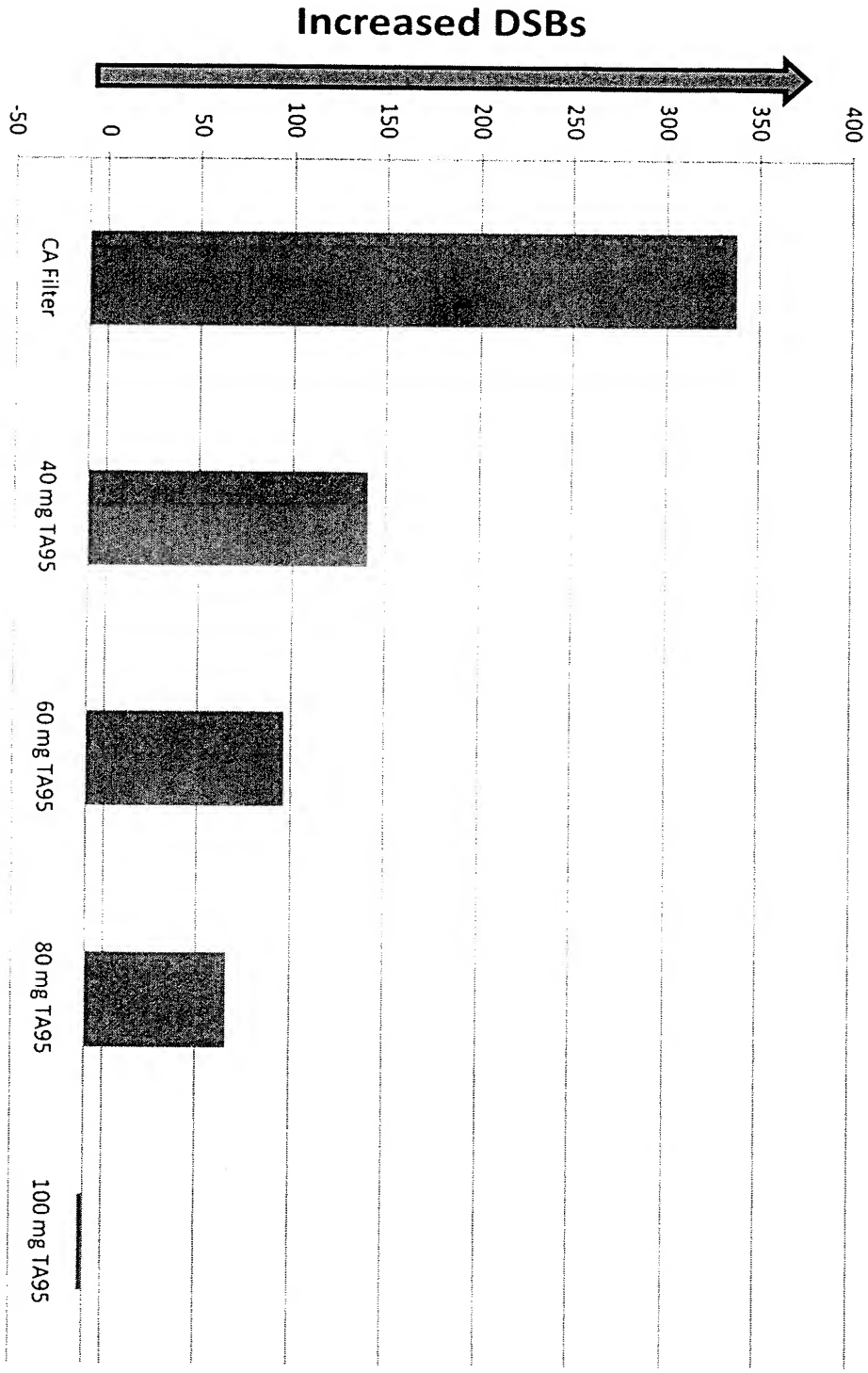

Increased DSBs
-50
0
50
100
150
200
250
300
350
400
CA Filter
40 mg TA95
60 mg TA95
80 mg TA95
100 mg TA95
Carbon with 10:90 Flue cured to Burley

FIGURE 3

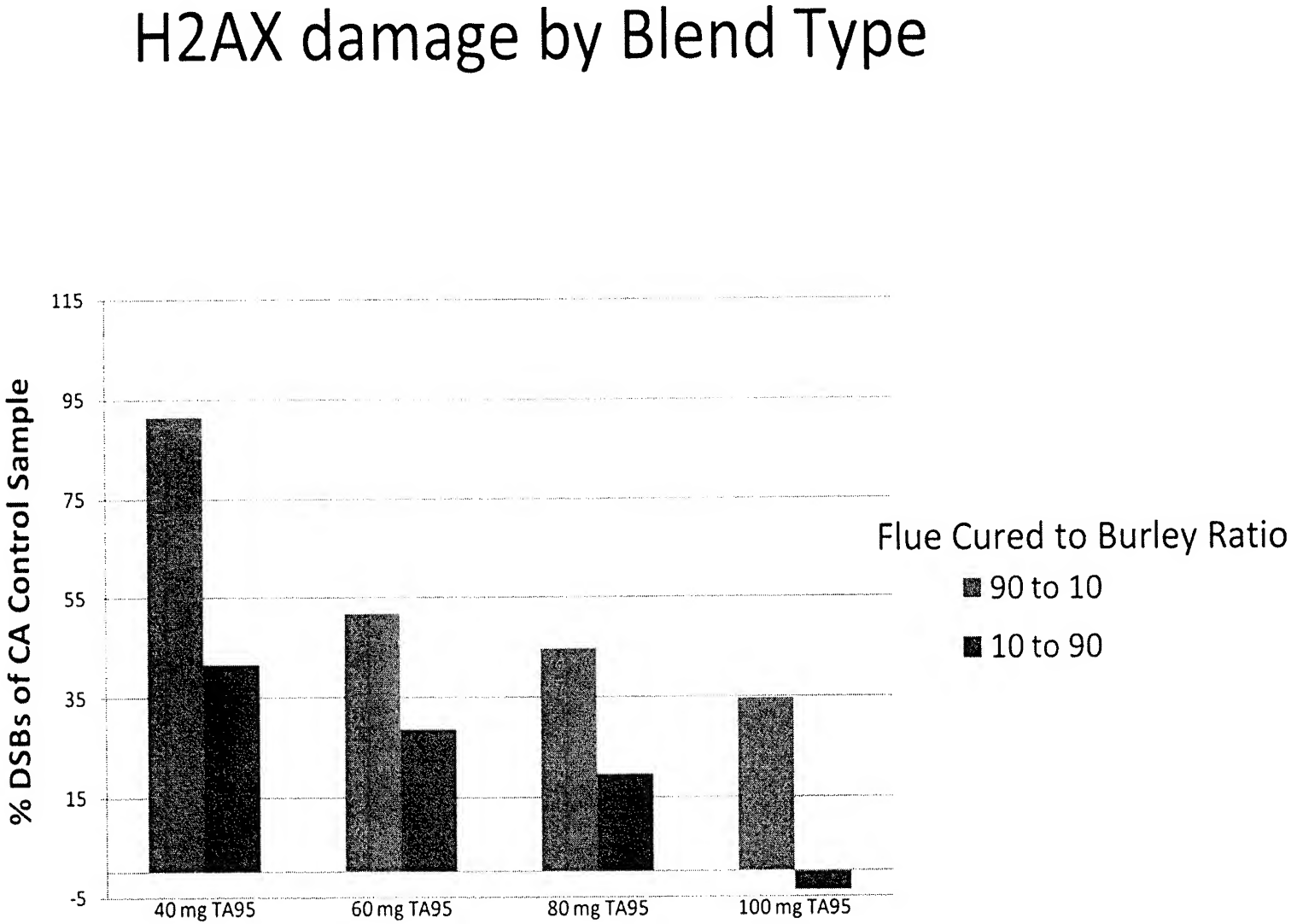
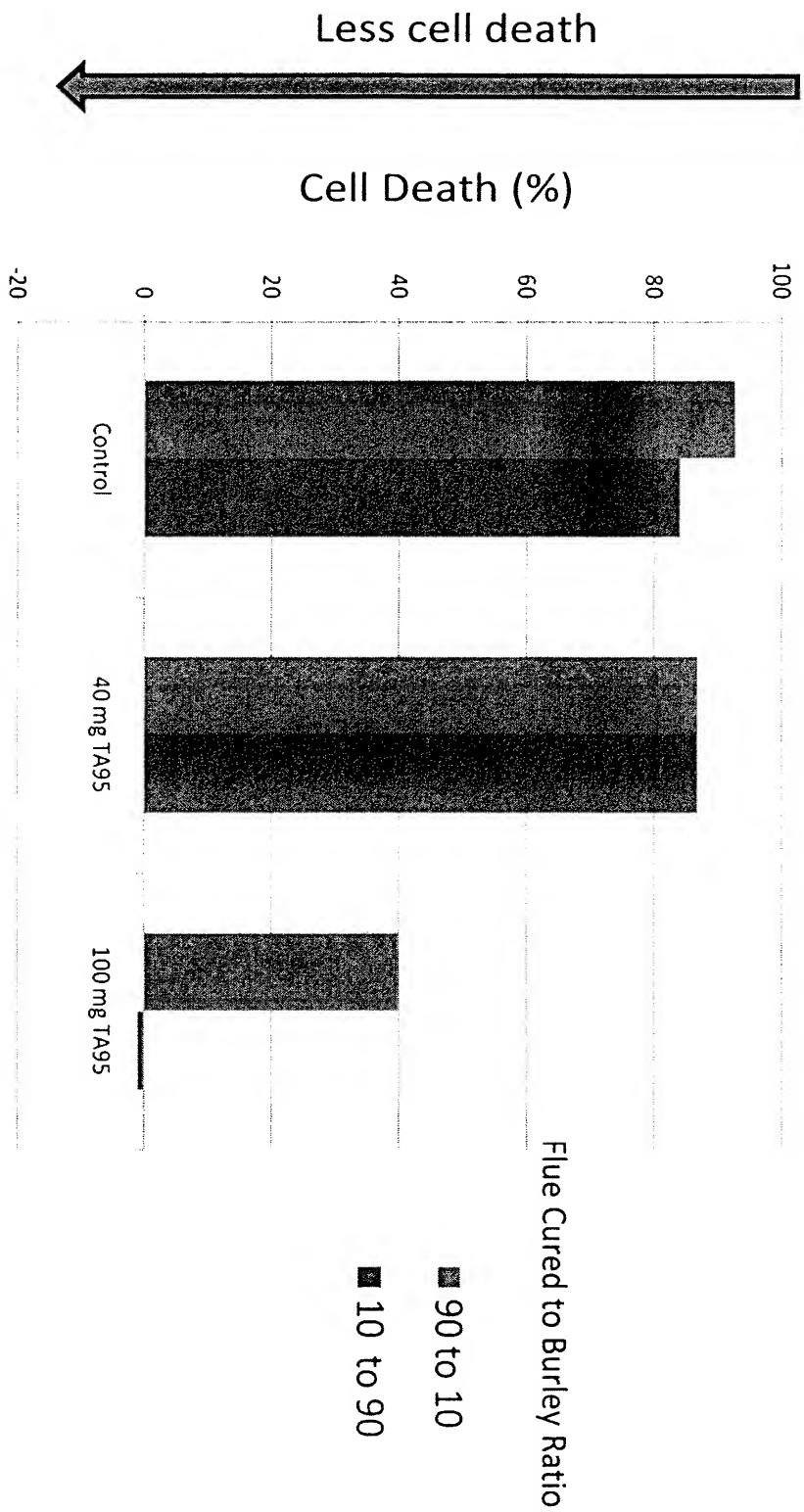

H2AX damage by Blend Type
% DSBs of CA Control Sample
115
95
75
55
35
15
-5
40 mg TA95
60 mg TA95
80 mg TA95
100 mg TA95
Flue Cured to Burley Ratio
90 to 10
10 to 90

FIGURE 4



Cloning Efficiency by Blend Type

FIGURE 5

H2AX Synergy Data for Carbon + Resin

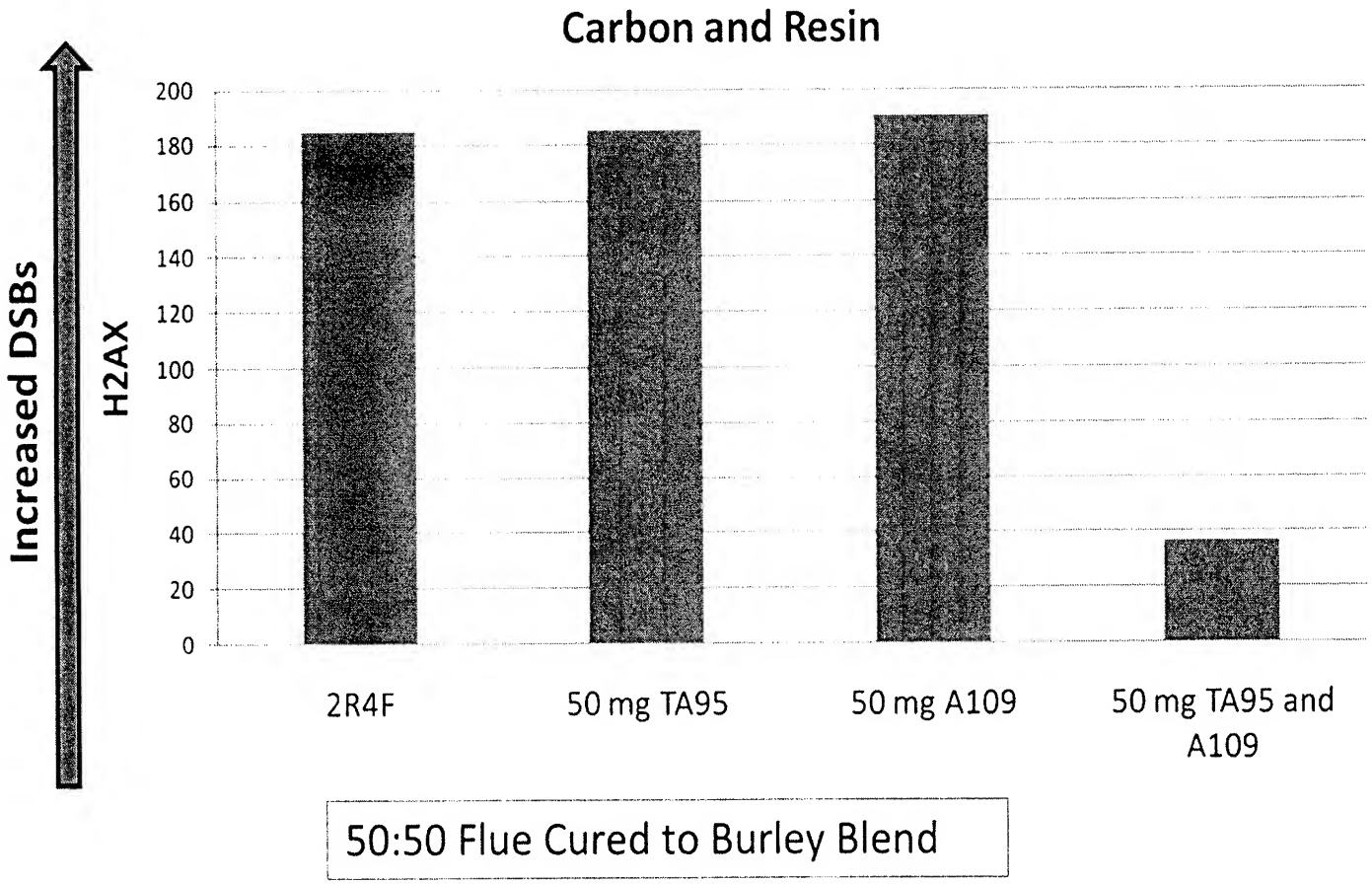


FIGURE 6

Clonogenic Synergy Data for Carbon + Resin

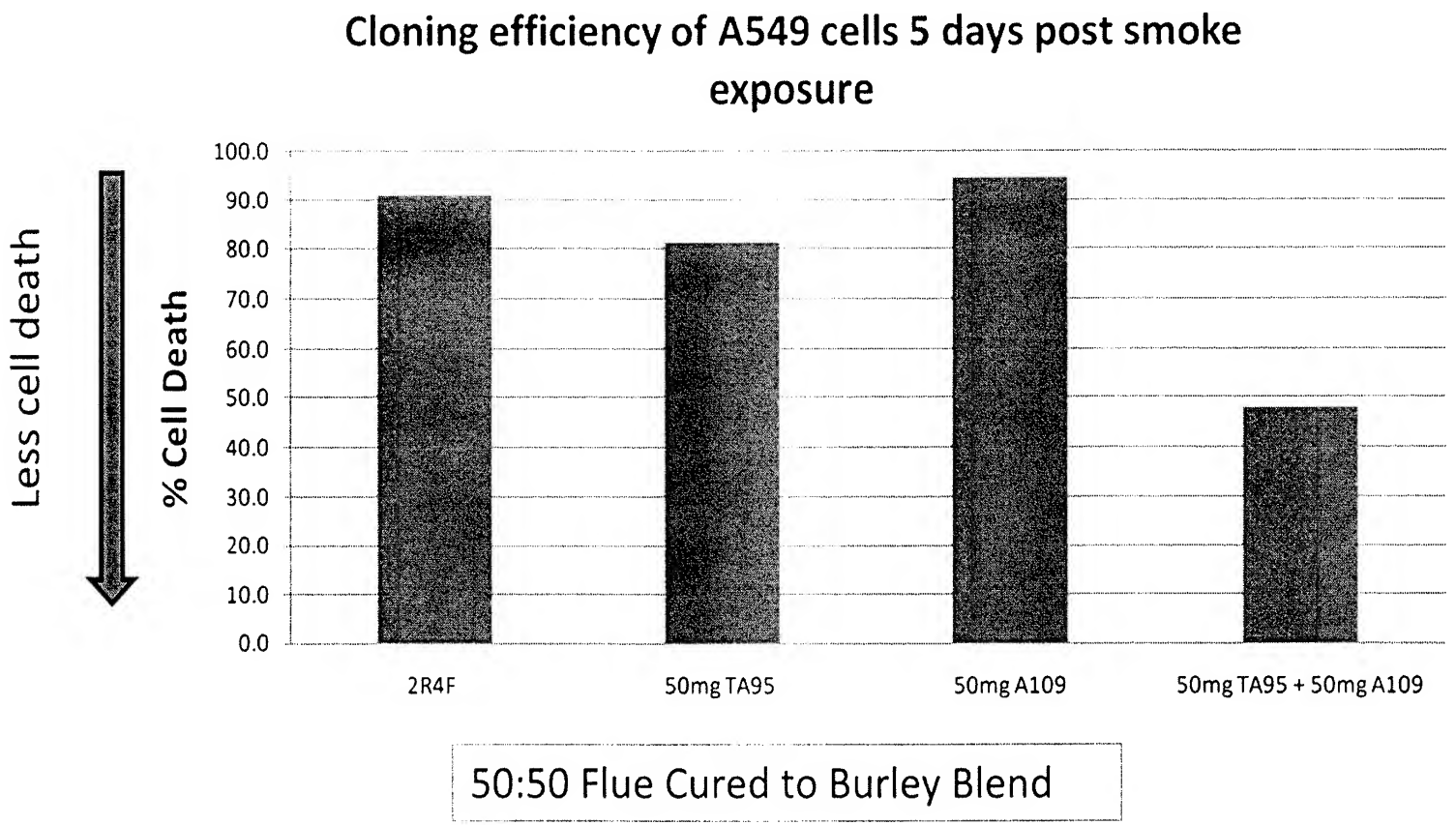


FIGURE 7

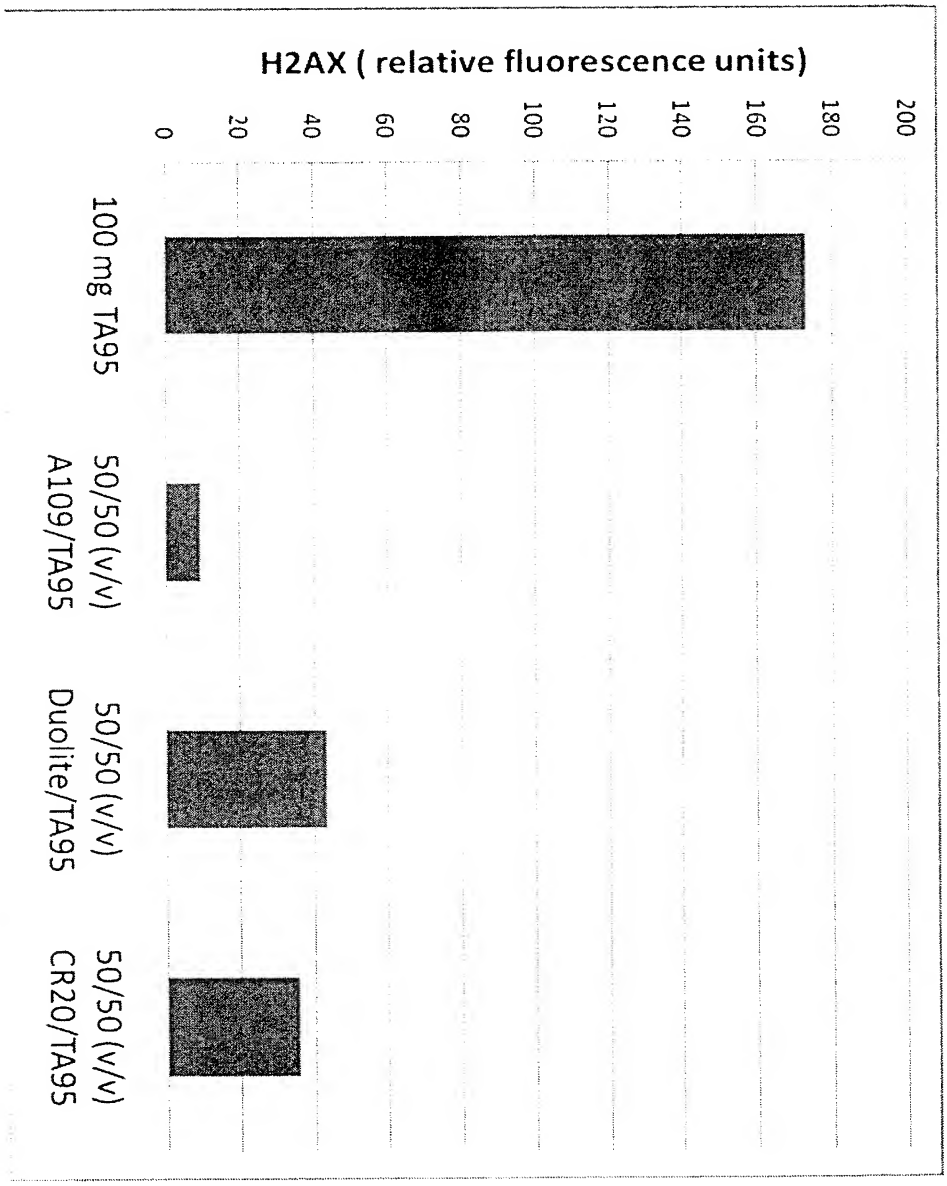

H2AX (relative fluorescence units)
0
20
40
60
80
100
120
140
160
180
200
100 mg TA95
50/50 (v/v) A109/TA95
50/50 (v/v) Duolite/TA95
50/50 (v/v) CR20/TA95

FIGURE 8

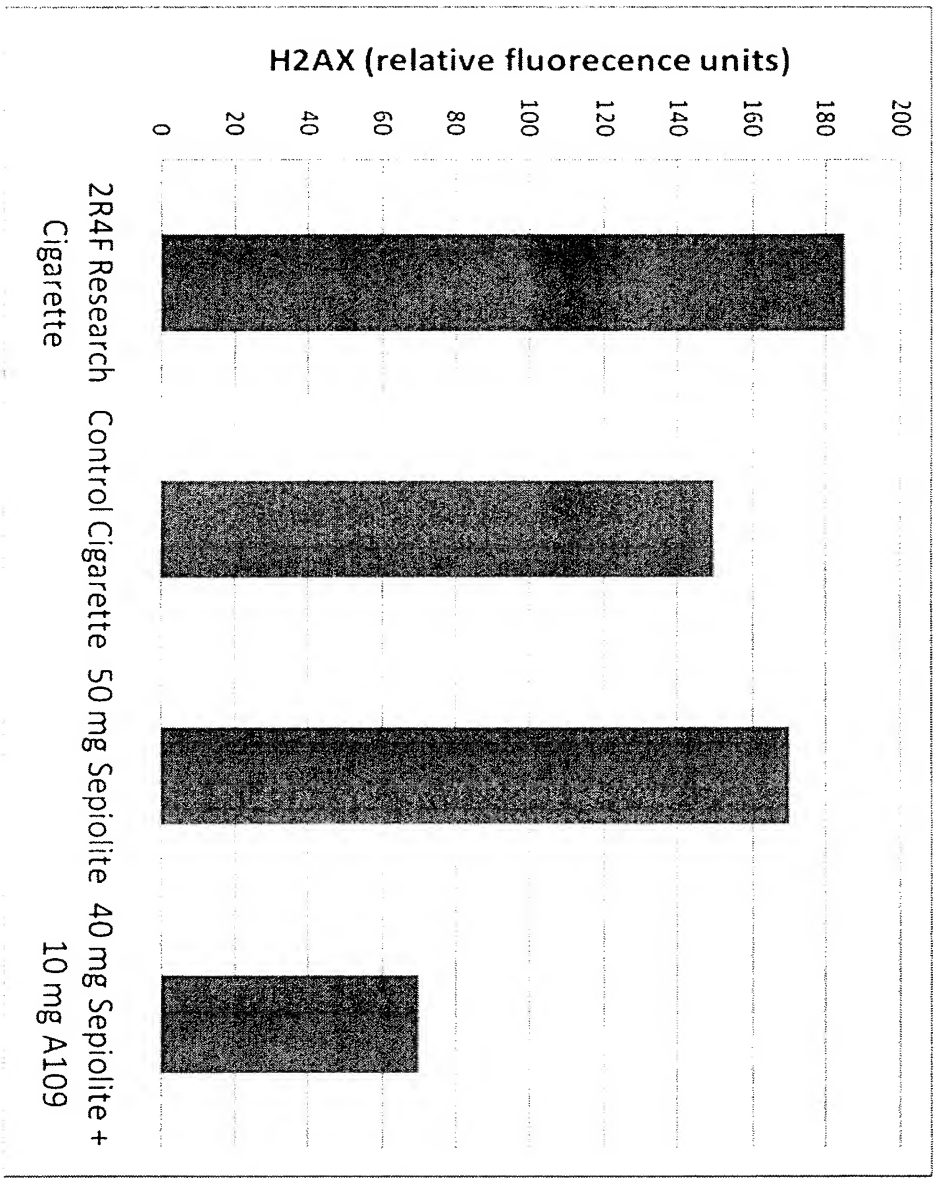

H2AX (relative fluorecence units)
0
20
40
60
80
100
120
140
160
180
200
2R4F Research Cigarette
Control Cigarette
50 mg Sepiolite
40 mg Sepiolite + 10 mg A109

FIGURE 9